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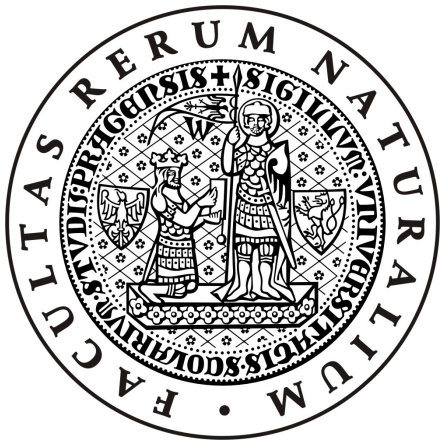

Mgr. Lucie Hemrová

Dynamika druhů fragmentovaných suchých trávníků na úrovni krajiny

Dynamics of species of fragmented dry grasslands at the landscape scale

Disertační práce
Ph.D. Thesis

Školitelka/Supervisor: Doc. RNDr. Zuzana Münzbergová, Ph.D.

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Abstract

Past extensive changes in land use have resulted in fragmentation of species habitats. Changes in landscape structure have provoked discussion about the prospects of species of fragmented habitats in the future agricultural landscape. Landscape dynamics of plant species is basically a result of extinction and colonization rates. Many studies have confirmed the former expectation that a number of habitats suitable for a plant species in a landscape stay unoccupied. The differences in species distribution and proportion of suitable habitats occupied by a given species are hypothesized to be due to the area and isolation of suitable habitats and colonization and survival ability of a given species. To understand species dynamics in a changing landscape, first we have to reveal the relationships between the distribution of species and their proportion of occupied habitats, habitat age, habitat configuration and species colonization and survival ability. After the complete understanding of the determinants of species dynamics we will be able to predict reliably species prospect in the future. The very basic aim of this thesis was to reveal the determinants of landscape dynamics of dry grassland species in terms of their distribution and frequency in the landscape.

The importance of correct identification of habitats suitable for a species in studies exploring determinants of species dynamics and distribution is obvious. There is, however, no single approach that would guarantee correct identification of habitats suitable for a species. In the **Chapter 1** I demonstrated a potential advantage of parameters derived from species composition and recommended their inclusion among habitat characteristics commonly used in species distribution models.

In **Chapter 2** I confirmed the hypothesis that isolation based on the number of target species in source habitats can explain colonization of focal abandoned fields by dry grassland species better than isolation based on the area of the habitats. I also confirmed that abandoned fields in the study landscape were successfully colonized by a large set of dry grassland species that are frequent in the dry grasslands. However, the rare species that are the object of interest in conservation biology occupied abandoned fields rarely. It suggests that abandoned fields can serve as potential habitats to only a part of the grassland species.

I also revealed that the presence of about 30% of tested dry grassland species is still affected by habitat configuration in the past (**Chapter 3**). Different species life-history traits relate to species dependence on historical vs. present habitat configuration. I consequently identified that the number of available suitable habitats is the major factor determining proportional habitat occupancy of these species (**Chapter 4**). The results also suggest differences in the quality of old and young habitats. The lack of suitable habitats is the main constraint in the dynamics of rare species. I assume that the only effective way to support the dynamics and distribution of rare species is to establish new grasslands with habitat quality similar to the quality of the old dry grasslands or to restore the quality of existing unsuitable habitats by introducing the appropriate management.

In **Chapter 5** I also found that factors affecting local species dynamics such as browsing by large herbivores can be very important for landscape-level dynamics of species with slow response to landscape changes. I suppose that these species can be potentially threatened by increasing number of large herbivores in the landscape.

Although I revealed some processes driving dynamics and distribution of dry grassland species in the landscape, there is still room for further research regarding the dominance of some species in dry grasslands and the prediction of future prospect of dry grassland species.

Introduction

Extensive changes in land use during the last few centuries have resulted in increased level of habitat fragmentation for many plant species (e.g., Cousins 2001; Jacquemyn et al. 2003; Hérault and Honnay 2005; Piessens et al. 2005; Chýlová and Münzbergová 2008; Johansson et al. 2008). The area of European semi-natural grasslands has been strongly reduced (e.g., Hamre et al. 2007; Johansson et al. 2008; Cousins 2009) and the cessation of former management practices has led to overgrowth of many existing localities by shrubs and trees (Lipský 1995; Poschlod and Bonn 1998; Eriksson et al. 2002; Luoto et al. 2003). These changes have provoked discussion about the effects of fragmentation on species dynamics and distribution (Eriksson et al. 1995; Fischer and Stöcklin 1997; Bastin and Thomas 1999; Dupré and Ehrlén 2002; Mildén et al. 2006; Cousins 2009).

Dynamics of plant species in landscape are result of extinction and colonization rates (e.g., Harrison 1991; Eriksson 1996; Husband and Barrett 1996; Ehrlén and Eriksson 2000; Münzbergová et al. 2005). Many studies have shown that a number of habitats suitable for a plant species in a landscape stay unoccupied (e.g., Turnbull et al. 2000; Münzbergová 2004; Ehrlén et al. 2006). The differences in species distribution and the proportion of suitable habitats occupied by a given species are hypothesized to be due to the configuration of suitable habitats on the one side (e.g., Levins 1969; Eriksson et al. 1995; Bastin and Thomas 1999; Bruun 2000; Dupré and Ehrlén 2002; Jacquemyn et al. 2003) and to species colonization and survival ability on the other side (e.g., Eriksson and Jakobsson 1998; Piessens et al. 2005; Tremlová and Münzbergová 2007; Lindborg et al. 2012).

Habitat configuration and its changes can affect both extinction of local populations and colonization of available suitable habitats. Larger habitats can host larger populations which are more resistant to the negative effects of demographic and environmental stochasticity (e.g., Fischer and Stöcklin 1997; Matthies et al. 2004; Münzbergová 2006). Habitat size is also important for colonization rates as larger habitats are better accessible to species. In addition, colonization rates are also affected by habitat connectivity (Levins 1969; Hanski and Gyllenberg 1993). Current habitat area and connectivity have been repeatedly shown to affect species occurrence (e.g., Eriksson et al. 1995; Bruun 2000; Dupré and Ehrlén 2002; Jacquemyn et al. 2003, Piessens et al. 2005; Lindborg et al. 2012). In contrast, there is also a lot of species for which the current habitat configuration has not been proven to be significant. Some of these species can, however, reflect historical habitat configuration due to dispersal limitation (Herben et al. 2006; Münzbergová et al. 2013) and extinction debt (Eriksson 1996). Such disequilibrium dynamics are very likely in many species of European agriculture landscape as a result of rapid landscape changes and slow species response to these changes.

A useful approach for understanding species dynamics in a changing landscape is to analyze the relationships among species distribution or proportional habitat occupancy, habitat configuration, habitat age and species colonization and survival ability (e.g., Verheyen et al. 2004; Kolb and Diekmann 2005; Adriaens et al. 2006; Endels et al. 2007; Tremlová and Münzbergová 2007; Schleicher et al. 2011; Koyanagi et al. 2012). Colonization and survival ability are expressed by a wide range of life-history traits such as seed mass, seed production, dispersal mode, plant height and seed bank longevity (e.g., Murray et al. 2002; Van der Veken et al. 2007; Kleyer et al. 2008).

Several studies have also used various models to understand species dynamics and predict species prospect (e.g., Vellend 2003; Verheyen et al. 2004; Münzbergová et al. 2005; Mildén et al. 2006). Such approach enables to manipulate the availability of suitable habitats and the dispersal and survival ability of a given species and follow their importance to species landscape-level dynamics.

Recognition of the habitats suitable for a species is fundamental for all studies concerning the determinants of species dynamics. Habitats suitable for a plant species can be recognized using a wide range of direct and indirect methods. The most common direct approach involves introduction of a species by sowing its seeds in occupied and unoccupied habitats (e.g., Ehrlén and Eriksson 2000; Turnbull et al. 2000; Münzbergová 2004; Ehrlén et al. 2006; Mildén et al. 2006; Moore and Elmendorf 2006). While this approach is considered useful in many cases, it has several limitations (time-consuming method requiring a lot of seeds and replicates) that lead to favoring indirect approaches over direct ones.

Indirect methods predict habitat suitability using the characteristics of both the habitats occupied and unoccupied by the species (e.g., Bastin and Thomas 1999; Thuiller et al. 2003; Münzbergová and Herben 2004; Liu et al. 2005; Moore and Elmendorf 2006; Wichmann et al. 2008). Compared to the direct approach, the indirect one requires less field work because a field survey is done only once and a lot of data on habitat characteristics can be obtained from maps. Nevertheless, a major weakness of the indirect approach is the assumption that target species occur primarily in suitable habitats. This assumption can result in imprecise predictions for species forming many remnant populations due to extinction debt. Although both direct and indirect approaches have been used increasingly in recent years, the predictive power of the direct cultivation experiments and indirect models based on different types of data have not been compared.

In this thesis I present a series of papers aimed at understanding species dynamics and distribution in a changing landscape. Specifically, I aim at (i) comparing methods identifying habitats suitable for a species (**Chapter 1**), (ii) estimation of the role of isolation in the colonization of abandoned fields by grassland species (**Chapter 2**), (iii) identification of species traits related to species dependence on historical vs. current habitat configuration (**Chapter 3**), (iv) identification of the determinants (landscape and species traits) of proportional habitat occupancy observed in the current landscape (**Chapter 4**) and (v) exploring the importance of browsing by large herbivores to the landscape-level dynamics of species with slow responses to landscape changes (**Chapter 5**).

All individual studies described in **Chapters 1-5** are situated in the Czech Republic and focus on semi-natural grasslands in agricultural landscape. Dry acidic grasslands, primary *Hyperico perforati-Scleranthion perennis* and *Koelerio-Phleion phleoidis* (Chytrý et al. 2001), are the target vegetation in the methodological study of **Chapter 1** (that compares the ability of different approaches to identify the habitats suitable for a species). This research was situated near Horažďovice in southwest Bohemia where dry acidic grasslands are typical of the landscape.

The studies in **Chapters 2-5** were situated near Litoměřice in northern Bohemia. Calcareous dry grasslands (alliance *Bromion erecti*, Ellenberg 1988) are typical of this landscape. These grasslands form distinct localities surrounded mainly by large agricultural fields and host several species endangered in the Czech Republic (e.g., *Coronilla vaginalis*, *Gentiana cruciata*, *Globularia elongata*, *Linum flavum* and *Scorzonera hispanica*; nomenclature follows Tutin et al. (1964–1983)). However, the area is under no formal protection. Some of the dry grasslands are grazed or mown, but most of the dry grasslands are currently not managed which leads to slow overgrowing of dry grasslands by shrubs and trees and to their degradation and loss. In addition to the habitat loss caused by succession at the localities, some of the localities are occasionally completely destroyed by ploughing or construction. Dramatic land use changes have been observed in the landscape (Chýlová and Münzbergová 2008). Although a part of the grasslands has been continuous more than 60 years, a larger part of dry grasslands has been established on abandoned fields between 1950s and 1980s. New grassland habitats have also appeared after 1980s. The research in **Chapter 2-5** benefits from and continues the previous research performed on these dry grasslands in the landscape (Münzbergová 2004;

Münzbergová 2006; Tremlová and Münzbergová 2007; Chýlová and Münzbergová 2008; Münzbergová and Plačková 2010).

Main results

In **Chapter 1** I focus on the identification of suitable habitats by different approaches. I compare the predictive power of distribution models for a short-lived herbaceous plant, *Jasione montana* L. I developed models based on the environmental characteristics of the habitats, on the species composition of the habitats, or on both of these types of data. I also established a cultivation experiment. The predictions of the different models are compared with the results of the cultivation experiment.

I found that the models based on the species composition of the habitats performed better than the model based only on environmental characteristics. The models also differed significantly in the unoccupied habitats they identified as suitable. The most accurate (highest AUC value) was the model based on both environmental characteristics and species composition. This model also significantly explained both the presence/absence and the abundance of the target species in the cultivation experiment. As there was a variation in the occurrence of the species in the cultivation unexplained by this model, I assumed that the predictions of this model, in spite of the high AUC values, were inaccurate for at least some habitats.

Although the results of this study are species and landscape specific, it demonstrates that assembling data on both environmental characteristics and species composition of habitats is likely to be useful for predicting habitat suitability at a landscape scale. The models with AUC between 0.7 and 0.9 are considered as useful for some applications (Swets 1988). This study, however, demonstrates that such an AUC value is not a guarantee that a model's prediction is reliable because a cultivation experiment may provide different results. Therefore, when identifying habitats that are suitable for a species (e.g., for the purpose of a metapopulation study), the results should be subjected to a sensitivity analysis.

In **Chapter 2** I explore the importance of habitat characteristics and isolation of abandoned fields for their colonization. Abandoned fields are perceived as potential habitats for species of threatened semi-natural dry grasslands. However, information is lacking regarding how the spontaneous colonization of abandoned fields depends on the broader spatial context.

In this chapter, the occurrence of 87 target species in 46 abandoned fields and 339 dry grasslands is used to test the effect of the isolation of abandoned fields on the success of colonization of dry grassland species from the surrounding grasslands. As a number of studies have not proven a positive relationship between patch area and species richness or population size (Eriksson et al. 1995; Kiviniemi and Eriksson 1999; Pärtel and Zobel 1999; Eriksson and Ehrlén 2001; Bruun 2005), two different measures were used to express the isolation of the abandoned fields. The isolation of the fields was calculated using the distance and area (I_A) or the distance and species richness (I_S) of source habitats. Moreover, two different values of both I_A and I_S differing in the measure of distance between the abandoned fields and the dry grasslands (edge-to-edge or center-to-center) were calculated. The effect of isolation on the number of dry grassland species on abandoned fields was explored both with and without habitat characteristics of the abandoned fields being used as covariates.

I_S always explained the number of species in the abandoned fields better than I_A. The different performance of I_A and I_S can be explained by the weak species–area relationship in the grasslands as some large grasslands host small number of target species probably due to different habitat quality unsuitable for a range of dry grassland species. The effect of

isolation also depended on using the habitat characteristics as covariates and became smaller or even non-significant with their use. The inclusion of habitat characteristics as covariates may considerably alter the conclusions regarding the effect of isolation, which might actually be overestimated when assessed separately. The method used for measuring distance was also crucial for the overall effect of isolation on species richness.

The conclusion of this chapter is that species richness is a better proxy of habitat importance in terms of propagule source than habitat area. This isolation measure is therefore suitable for studying the effects of landscape structure on species richness in landscapes with a weak species–area relationship.

In **Chapter 3** I answer the question which species traits are related to species dependence on habitat configuration in different time periods. Although a number of studies identify plant life-history traits responsible for species distribution in fragmented landscape, the effect of individual traits responsible for species dependence on historical vs. current landscape configuration remains unclear. I identified to what extent present (2000s) and historical (1843, 1954 and 1980s) landscape configuration affected current species composition of dry calcareous grasslands. Moreover, I used traits related to dispersal, survival, growth and habitat preference to explain the dependence of 60 dry grassland species on present and historical habitat configuration.

I found that the composition of dry calcareous grasslands was affected by both area and connectivity in all time periods. Detailed analyses of species occurrences revealed that 69 % of species under study depended positively on habitat configuration, but almost half of them (44 %) depended only on habitat configuration in the past suggesting that many dry grassland species are not in equilibrium with the current landscape. Endozoochorous fast-growing species with higher seed bank longevity and requirements to nutrients were positively associated with habitat area and connectivity in the last 30 years. In contrast, epizoochorous slow-growing species with lower seed bank longevity and requirements to nutrients but higher requirements to light were positively associated with habitat area and connectivity up to 1954.

These results indicate that the relationship between species traits and landscape structure strongly depends on the quality of habitats in specific time periods as well as on other factors such as specific type of land use. Predictions of which species will profit from future changes in the landscape will thus need to carefully consider not only the overall change in the landscape structure but also details of the processes accompanying this change such as effects of land-use and its changes on species distribution.

In **Chapter 4** I continue to explore the determinants of dynamics of the dry grassland species in the landscape. I use life-history traits of the species and landscape characteristics of their suitable habitats to find the determinants of differences in habitat occupancy (i.e. the proportion of occupied habitats). A number of studies have showed the effect of life-history traits on habitat occupancy. Only few studies, however, focus on the effect of the mean age and the overall number of suitable habitats of a species on its habitat occupancy, although both age and isolation of individual habitats were found to have an impact on species distribution. The relationship among the mean age of habitats suitable for a given species, the overall isolation of habitats suitable for the given species, species life-history traits and species habitat occupancy remains unclear.

I identified suitable habitats for a set of dry grassland species in current landscape. Based on the information from old maps (1843, 1954 and 1980s) I estimated the age of each current dry grassland habitat. The mean age and the total number of suitable habitats of each species and traits related to dispersal, survival, growth and habitat preference were then used to explain differences in habitat occupancy among 60 dry grassland species.

I found seed bank longevity and the number of suitable habitats to have a positive effect on habitat occupancy, while the mean age of suitable habitats was found to have a negative effect on habitat occupancy. I also found negative relationship between number

and age of suitable habitats of the species. Species occupying younger habitats had higher specific leaf area and requirements to nutrients suggesting a difference in habitat quality between younger and older habitats.

These results indicate that the availability of suitable habitats is a major factor determining species habitat occupancy. The number of suitable habitats is, however, influenced by species traits such as species requirements to nutrients and specific leaf area; fast-growing competitive species have more suitable habitats as they include both older and younger habitats among those that are suitable for them. Although we identified seed bank longevity as the only important species trait explaining habitat occupancy, requirements to nutrients and specific leaf area affect habitat occupancy indirectly through their impact on the number of habitats suitable for the given species.

In **Chapter 5** I focus on the other factors that have an impact on species dynamics in landscape. Specifically, I simulate the dynamics of a target species in the landscape and explore to what extent the dynamics and prospect of the species in the landscape is affected by activities of large herbivores and occasional destruction of species' populations.

Models assessing the prospects of plant species at the landscape level often focus primarily on the relationship between species dynamics and landscape structure. However, the short-term prospects of species with slow responses to landscape changes depend on the factors affecting local population dynamics. In this study it is hypothesized that large herbivores may be a major factor affecting the short-term prospects of slow-responding species in the European landscape, because large herbivores have increased in number in this region in recent decades and can strongly influence local population dynamics.

The impact of browsing by large herbivores was simulated on the landscape-level dynamics of the dry grassland perennial polycarpic herb *Scorzonera hispanica*. A dynamic, spatially explicit model that incorporated the information on the location of patches suitable for *S. hispanica*, local population dynamics (matrices including the impact of large herbivores), initial population sizes and the dispersal rate of the species was used. Simulations were performed relating to the prospects of *S. hispanica* over the next 30 years under different rates of herbivory (browsing intensity) and varying frequencies of population destruction (e.g., by human activity).

Although a high rate of herbivory was detected in most populations of *S. hispanica*, current landscape-level dynamics of *S. hispanica* were approximately in equilibrium. A decline or increase of over 20% in the herbivory rate promoted rapid expansion or decline of *S. hispanica*, respectively. This effect was much stronger in the presence of population destruction. In this paper I therefore assume that browsing by large herbivores can have a dramatic effect on the landscape dynamics of plant species. Changes in the density of large herbivores and the probability of population destruction should be incorporated into models predicting species abundance and distribution.

Conclusions and future directions

The importance of a correct identification of habitats suitable for a species in studies exploring the determinants of species dynamics and distribution is obvious. In **Chapter 1** I summarize the advantages and disadvantages of direct and indirect approaches of identification of suitable habitats. The comparison of different approaches indicates that it is difficult to preselect habitat characteristics that are important for species presence; I found that habitat characteristics commonly used in species distribution models can prove insufficient for predicting species distribution. The results also demonstrate the potential advantage of parameters derived from species composition. Therefore I recommend including the parameters derived from species composition among the habitat characteristics commonly used in species distribution models, although their different

importance for the distribution of different species has to be kept in mind. Of the parameters derived from species composition, I highly recommend using Beals index because of its small number of degrees of freedom along with its high explanatory power. Beals index can be calculated from both phytosociological relevés and species lists. As recording species composition can be difficult in large areas, the possibility of using the existing databases and past records of species occurrences to compute Beals index should be considered; however, the predictive power of this approach should be estimated when applied to large areas.

In **Chapter 2** I confirmed the hypothesis that isolation based on the number of target species in source habitats can explain the colonization of focal abandoned fields by dry grassland species better than isolation based on the area of the habitats. It indicates a complicated relationship between the area of a habitat and its quality in terms of propagule source. Inclusion/omission of habitat characteristics in the analyses influenced the results. It indicates that habitat characteristics of focal abandoned field and surrounding source habitats are not independent on each other. Therefore using tests both with and without inclusion of habitat characteristics enables better understanding of factors that influence species richness. I confirmed that abandoned fields in the study landscape were successfully colonized by a large set of dry grassland species that are frequent in the dry grasslands. However, the rare species that are the object of interest in conservation biology occupied abandoned fields rarely. It suggests that abandoned fields can serve as potential habitats to only a part of the grassland species. Therefore the future conservation and management of those grasslands hosting the rare species becomes more important for the maintenance of the current species richness of the dry grasslands in the future.

Chapter 3 continues on previous research in exploring the determinants of species distribution. In this study I revealed that about 30% of dry grassland species under this study did not depend on habitat configuration at the present, but only on habitat configuration in the past. It suggests that the distribution of these grassland species is not in equilibrium with the current landscape. The reasons for species dependence on past vs. current habitat configuration can be found in species life-history traits. While epizoochorous slow-growing species with lower seed bank longevity and requirements to nutrients but higher requirement to light primarily colonize the old habitats and occur on these habitats for a long time, the endozoochorous fast-growing species with higher seed bank longevity and requirements to nutrients colonize the younger habitats with different habitat quality.

Exploring the determinants of habitat occupancy of the dry grassland species in **Chapter 4** is the final piece in the puzzle of species dynamics in the landscape. The number of available suitable habitats is the major factor determining species habitat occupancy. The results of this study suggest differences in the quality of old and young habitats. Species occupying young habitats are those having high number of suitable habitats in the landscape. These species are fast-growing with higher competitive ability and they can thus succeed on current grasslands exposed to slow habitat degradation and succession. The only effective way to support the dynamics and distribution of rare species is to establish new grasslands with habitat quality similar to the quality of the old dry grasslands. As the grasslands occur in typical agricultural landscape, the possibility to establish new grasslands is strongly limited. Another possibility to increase the connectivity of the habitats suitable for rare species is to restore the quality of some unsuitable habitats by introducing the appropriate management.

Although the changes in the availability of suitable habitats are the major factor influencing habitat occupancy of dry grassland species in the landscape, other factors such as browsing by large herbivores could have a dramatic impact on their landscape-level dynamics. In **Chapter 5** I confirmed the hypothesis that the increasing number of large herbivores in the landscape could reduce the abundance of those species with slow

responses to landscape changes that are negatively influenced by browsing of the herbivores. The number of large herbivores, such as roe deer, has been increasing in the agricultural European landscape in recent decades. There is no reason why we should expect the stagnation of this increase in the near future. Therefore some species can get under serious pressure of browsing by the large herbivores. The future studies estimating the prospect of these species in a landscape should thus concern the role of the herbivores in the species landscape-level dynamics.

In this work I answered some of the questions about species dynamics and distribution in the landscape. There are, however, other things that remain unexplained. Dry grasslands in the landscape are often dominated by one out of four species (*Anthericum ramosum*, *Brachypodium pinnatum*, *Bromus erectus* or *Inula salicina*). The cause of this dominance among the dry grasslands is hidden for now. The dominance of one particular species on a habitat can be caused by its different habitat conditions in the time of colonization by the species or by subsequent positive feedbacks between the species and its environment. Currently I collaborate with Jana Knappová on field experiments that will explore the determinants of species dominance on the dry grasslands.

In the future I would like to focus also on another unexplored field in species dynamics. I want to try to predict the dynamics of more than one species in the landscape using the detailed information on species characteristics of only a few species. First, it is however necessary to identify the importance of local dynamics, dispersal ability and availability of suitable habitats in the model of a species landscape-level dynamics. Then I will link the results of simulations of the prospect of a species with the few species of which local dynamics, dispersal ability and the number of suitable habitats are known. Consequently I will identify the relationships between species life-history traits and local dynamics, dispersal ability and the number of suitable habitats in these few species. These relationships could enable me to assume the prospect of a species based on its life-history traits. Such an approach could also allow considering the expected future changes in landscape and therefore answer the question about the future prospect of a dry grassland species in the changing landscape.

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Chapter 1

Identification of suitable unoccupied habitats: direct versus an indirect approach

Lucie Hemrová and Zuzana Münzbergová

Abstract

Identification of habitats suitable for a species is a key task when studying landscape dynamics. Direct (cultivation experiments) or indirect (species distribution models) methods are employed to identify both suitable but currently unoccupied habitats and habitats that are unsuitable yet occupied (remnant populations). Although both direct and indirect approaches have been used increasingly in recent years the predictive power of cultivation experiments and of models based on different types of data have not been compared. In the present study, we compare the predictive power of distribution models for a short-lived herbaceous plant, *Jasione montana* L. Our models are based on the environmental characteristics of the habitats, on the species composition of the habitats, or on both of these types of data. The predictions of the different models were compared (using AUC values) with the results of our cultivation experiment. We found that the models based on the species composition of the habitats performed better than the model based only on environmental characteristics. The models also differed significantly in the unoccupied habitats they identified as suitable. The most accurate was the model based on both environmental characteristics and species composition. This model also significantly explained both the presence/absence and abundance of *J. montana* individuals in the cultivation experiment. Nevertheless, the variation in occurrence of *J. montana* in the cultivation experiment explained by this model was below 50%. We therefore assume that the predictions of this model, in spite of the high AUC values, were inaccurate for at least some habitats. The results of this study are species and landscape specific, so they cannot be generalized. Our study, however, demonstrates that assembling data on both environmental characteristics and species composition of habitats is likely to be useful for predicting habitat suitability at a landscape scale. This study also demonstrates that a high AUC value is not a guarantee that a model's prediction is reliable because a cultivation experiment may provide different results. When identifying habitats that are suitable for a species (e.g., for the purpose of a metapopulation study), the results should be subjected to a sensitivity analysis.

Keywords: Beals index, community-composition data, distribution patterns, dry acidic grasslands, Ellenberg indicator values, patch occupancy, seed addition experiment

Introduction

Identification of habitats suitable for a species is an important task when studying its landscape dynamics (e.g., Ehrlén and Eriksson 2000; Münzbergová 2004; Guisan and Thuiller 2005; Herben et al. 2006). By identifying suitable habitats, it is possible to recognize habitats occupied due to extinction debt (Eriksson 1996). Analogically, we can identify habitats that are unoccupied because of the dispersal limitations of species (e.g., Ehrlén and Eriksson 2000; Turnbull et al. 2000; Münzbergová 2004).

Habitats suitable for a plant species can be recognized using a wide range of direct and indirect methods. The most common direct approach involves introduction of a species by sowing its seeds in occupied and unoccupied habitats (e.g., Ehrlén and Eriksson 2000; Turnbull et al. 2000; Münzbergová 2004; Ehrlén et al. 2006; Mildén et al. 2006; Moore and Elmendorf 2006). While this approach is considered useful in many cases it has several limitations. Firstly, habitat unsuitability can be proved only in the later stages of plant development (e.g., Turnbull et al. 2000; Ehrlén et al. 2006). Sowing experiments should therefore be continued until the plants have matured and reproduced, which may take up to several decades in the case of a perennial herbaceous plant (Ehrlén and Lehtilä 2002). Secondly, due to demographic stochasticity, a high initial number of seeds should be used in every sowing experiment. Thirdly, the success of sowing experiments depends on the year of establishment due to among-year variation in the species' survival (e.g., caused by weather conditions). Among-year variation may thus result in misleading results, so sowing experiments should be carried out repeatedly. Fourthly, sowing experiments cannot be done using invasive or endangered species. Finally, sowing experiments are time-consuming and require a lot of fieldwork. In many cases, these disadvantages result in indirect approaches being favoured over direct ones.

Indirect methods predict habitat suitability using the characteristics of both the habitats occupied and unoccupied by the species (e.g., Bastin and Thomas 1999; Thuiller et al. 2003; Münzbergová and Herben 2004; Liu et al. 2005; Moore and Elmendorf 2006; Wichmann et al. 2008). Compared to the direct approach, the indirect one requires less field work because a field survey is done only once. In addition, a lot of data on habitat characteristics can be obtained from various maps. One clear disadvantage of indirect methods is that the resulting predictions can depend on the frequency of species' occurrence (e.g., Gu and Swihart 2004; Segurado and Araújo 2004). Nevertheless, this problem can be solved, as suggested for example by Osawa et al. (2011). Another problem is that the species are assumed to occur primarily in suitable habitats. This assumption can result in imprecise predictions for species forming many remnant populations. The occurrence of remnant populations is more likely in long-lived species and species with extensive clonal growth (Eriksson 1996). Using a short-lived species with rapid responses to habitat deterioration can thus avoid this problem. And finally, the quality of a model depends on the right selection of parameters. This problem can be partly overcome by using the species composition of habitats to assess their suitability, since recorded species may reflect the same habitat characteristics that are also important for the target species (e.g., Münzbergová 2004; Münzbergová and Herben 2004; Guisan and Thuiller 2005; Ehrlén et al. 2006; Mildén et al. 2006; Moore and Elmendorf 2006). Species composition can, however, result from dispersal limitation, not habitat limitation, which limits the utility of the method. Similarly, the use of species composition is restricted in many species with remnant populations at the sites studied.

Species distribution models are usually developed using a range of abiotic and biotic environmental predictors. The predictors are usually obtained from maps or databases for larger study areas and from field data collected for smaller study areas (i.e. up to ca 100 km^2). Abiotic predictors include parameters characterizing soil and geology (e.g., Ehrlén and Eriksson 2000; Thuiller et al. 2003; Guisan et al. 2007; Wichmann et al. 2008), habitat

configuration (Bastin and Thomas 1999), topography and bioclimatic conditions (e.g., Thuiller et al. 2003; Liu et al. 2005; Osawa et al. 2011; Rupprecht et al. 2011), habitat history and land use (Bastin and Thomas 1999; Rouget et al. 2001). The biotic predictors include parameters of particular species or vegetation such as occurrence, cover, height or leaf type (e.g., Münzbergová 2004; Guisan et al. 2007). In contrast to these predictors, detailed information on species composition of habitats has not often been exploited for making predictions about species occurrence (but see e.g., Dupré and Diekmann 1998; Münzbergová and Herben 2004; Tájek et al. 2011; Hemrová et al. 2012). It has been suggested that parameters derived from species composition might be better predictors of species occurrence than environmental predictors (e.g., Dupré and Diekmann 1998; Münzbergová 2004; Ehrlén et al. 2006; Elmendorf and Moore 2008). A comparison of the performance of models based (i) only on species composition, (ii) only on environmental parameters and (iii) both on species composition and environmental parameters with each other and with results of cultivation experiments is crucial for determining which method is best at predicting the suitability of habitats for species. Although all these approaches have been used repeatedly, no such comparison has been done.

In the present study, we therefore predict the presence of a species using indirect methods (i)–(iii) and compare the model predictions with the results of a cultivation experiment. We ask the following questions: (i) What is the influence of characteristics derived from species composition on model performance? (ii) Do models using different habitat characteristics detect the same suitable but unoccupied habitats? (iii) What is the correspondence between habitat suitability predicted using different habitat characteristics and the results of a cultivation experiment?

To answer these questions, we decided to estimate habitat suitability for the short-lived herbaceous plant *Jasione montana* L. By choosing a short-lived species, we reduced both the time necessary for following the cultivation experiment and the risk of encountering remnant populations. For a set of dry acidic grassland habitats in a particular area, we collected data on abiotic conditions and species composition. We developed models predicting habitat suitability using data on abiotic conditions, on species composition, or on their combination. In several habitats, we also established a cultivation experiment. The results of the cultivation experiment were subsequently compared with the results of the models predicting habitat suitability.

Methods

Species and area studied

Jasione montana L. (*Campanulaceae*) is a moderately frequent, short-lived, monocarpic, rosette forming herbaceous plant inhabiting dry grasslands in most of Europe (Tutin 1976). According to Parnell (1985) individual plants of *J. montana* can produce up to 4335 seeds. As its seeds are small and light [0.6 mm, 19.4 μg (Kleyer et al. 2008), V*term* = 0.7 $m.s^{-1}$ (L. Hemrová, unpublished data)], it is likely they are dispersed by wind. Experiments in which the seed was attached to animal fur (roe deer, wild boar and brown hare), however, indicate the seed could be dispersed via exozoochory (Hemrová, unpublished data). Sixty percent of the seeds is viable based on a mix of 1200 seeds from three populations germinated in a growth chamber under the following conditions: 12 hours light and 20°C, 12 hours dark and 10°C (L. Hemrová, unpublished data). Seeds mature from September to October, and some of them start to germinate shortly afterwards. This species has a persistent seed bank (Thompson et al. 1997) but most of its mature seeds germinate the following spring or summer (L. Hemrová, unpublished data). *Jasione montana* has a

scattered distribution in the study area. Most of the *J. montana* populations contained less than 50 flowering individuals and only one contained more than 100.

The study area (square and about 3 km^2; NW corner: 49°21'38"N, 13°39'24"E; SE corner 49°20'47"N, 13°41'4"E) is situated in a fragmented agricultural landscape in southwest Bohemia in the Czech Republic. Dry acidic grasslands hosting *J. montana*, primary *Hyperico perforati-Scleranthion perennis* and *Koelerio-Phleion phleoidis* (Chytrý et al. 2001) are typical of the landscape. The grasslands are surrounded by agricultural fields or partly by shrubs, forests or paths. In the study area, *J. montana* is restricted to these grasslands.

Data collection

We identified the locations of all the dry acidic grasslands in the study area (N = 122) in 2005–2006. These habitats contained similar vegetation and were surrounded by visually obvious barriers such as forests, shrubs, fields or paths. To increase the sample size, we added to the dataset six habitats hosting *J. montana* that were outside the study area (≤ 7 km). In total, we surveyed 128 dry acidic grassland habitats (ranging from one to 3260 m^2), 26 of which hosted *J. montana*. Habitat occupancy was detected based on presence of *J. montana* anytime between 2005 and 2010.

To assess habitat suitability for *J. montana*, we recorded both abiotic conditions and species composition in each habitat in 2005–2006. Abiotic parameters were chosen to represent commonly assembled habitat characteristics such as slope, potential direct solar irradiation (PDSI) or soil properties (e.g., Rouget et al. 2001; Thuiller et al. 2003; Wichmann et al. 2008). Data on 9 recorded abiotic parameters (Table 1) included: area (logarithm), slope, PDSI for the 21st day of the month from January to June (calculated using data on slope and aspect of given habitat; only the two least correlated were finally used), contact with a path (expressed as a percentage of habitat boundary with a path), presence of deep soil (>10 cm), shallow soil (< 5 cm), gravel and stones in a habitat (Table 1). Other habitat characteristics were calculated using species composition.

One phytosociological relevé of 9, 4 or 1 m^2, depending on the area of the habitat, was randomly placed and recorded in each habitat. Based on the species composition, we first calculated Beals index. Beals index expresses the probability of a species' presence in a habitat based on the number of joint occurrences with other species in a dataset (Beals 1984; Münzbergová and Herben 2004). We then calculated mean Ellenberg indicator values for light, temperature, moisture, nutrients, soil reaction and continentality (Ellenberg 1988, Table 1) for each habitat.

In late October 2007, we established a cultivation experiment in 21 habitats situated in the study area, three of which hosted several flowering individuals of *J. montana*. This proportion of occupied habitats corresponded to the habitat occupancy in the study area (i.e. 20 out of 122 habitats were occupied). We selected the habitats to cover the whole range of habitat quality in the area. Twelve plots (30 × 30 cm each) arranged in a block (4 × 3 plots) were placed > 1 m from the closest flowering *J. montana* individual to reduce the number of seedlings originating from natural seed rain and < 3 m from the recorded phytosociological relevé. Before sowing, we mechanically disturbed six plots by removing all above-ground vegetation. Three seed sources (originating from three external populations) were used in each block sown with seed, but the seeds were not mixed. Specifically, 300 seeds from one particular source were sown in one disturbed and 300 in one undisturbed plot in a block, so that six plots in a block were sown (3 sources × 2 plots). The remaining six plots were used as controls. The numbers of *J. montana* individuals in the cultivation experiment were recorded each September from 2008 to 2011.

Table 1. – List of the habitat characteristics used to develop predictive models of habitat suitability for *Jasione montana* using (i) abiotic parameters, (ii) Beals index, (iii) Ellenberg indicator values and (iv) a combination of habitat characteristics. R^2 and effect (+/-) is shown for characteristics included in the model (values significant at P = 0.05 are highlighted in bold). n indicates characteristics not included in the model; x indicates characteristics excluded from the test.

Habitat characteristics	Type	Models			
		Abiot	Beals	Ellenberg	Full
Abiotic parameters					
Area	continous	+ 0.030	x	x	n
Slope	continous	+ 0.029	x	x	+ 0.023
PDSI					
March	continous	n	x	x	n
June	continous	n	x	x	n
Contact with path	continous	**+ 0.037**	x	x	**+ 0.080**
Deep soil	binomial	n	x	x	- 0.020
Shallow soil	binomial	n	x	x	n
Gravel	binomial	+ 0.023	x	x	n
Stones	binomial	n	x	x	+ 0.024
Beals index	continous	x	**+ 0.433**	x	**+ 0.456**
Ellenberg indicator values					
Light	continous	x	x	+ 0.021	n
Moisture	continous	x	x	n	n
Soil reaction	continous	x	x	n	n
Nutrients	continous	x	x	**- 0.044**	n
Temperature	continous	x	x	**- 0.055**	n
Continentality	continous	x	x	**- 0.033**	n

Data analysis

To estimate habitat suitability, we tested for the effects of (i) abiotic parameters, (ii) Ellenberg indicator values and (iii) the combination of all habitat characteristics (including Beals index) on the presence of *J. montana*. Beals index was not used in a separate test, as its values directly represent the probability of the presence of *J. montana*. We used a generalized linear model (GLM) with a binomial distribution of the dependent variable (presence/absence of *J. montana*) in these tests. To simplify the models (see correlation matrix in Appendix 1), we used a step-wise bidirectional regression starting with the maximal model. We used the Akaike information criterion (AIC, Crawley 2002) to identify those habitat characteristics that best explained the presence of *J. montana*. We then calculated the probability of *J. montana* presence in individual habitats (Crawley 2002) for each developed model. In total, we had four models predicting *J. montana* presence: Beals index (model Beals), a model based on abiotic parameters (model Abiot), a model based on Ellenberg indicator values (model Ellenberg) and a model based on the combination of all habitat characteristics (model Full). To identify the habitats suitable for *J. montana*, we used the cut-off probability value in which the sum of sensitivity (i.e. the ratio of correctly predicted presences) and specificity (i.e. the ratio of correctly predicted absence) was maximized (Liu et al. 2005).

The performances of each model was evaluated by both calculating the area under the receiver operating characteristic curve (AUC, Swets 1988) and by comparing the suitability estimates with the results of the cultivation experiment. The receiver operating characteristic curve is obtained by plotting sensitivity vs (1–specificity) for various probability thresholds. The area under the curve may range from 0 for an inverse model, through 0.5 for a random model, to 1 for a perfect model. First, we tested the presence and

abundance of *J. montana* individuals in the cultivation experiment using a GLM with a binomial and Poisson distribution, respectively. Predicted probabilities of a model based on habitat characteristics were used as an independent variable. Since no natural regeneration of *J. montana* was observed in the control plots, we tested both the total number of individuals and the presence of any individual in a sown block in (i) 2008, (ii) 2009 and (iii) 2010 and (iv), the sum of flowering individuals in a sown block from 2008 to 2011, and the presence of any flowering individual in a sown block anytime between 2008 to 2011. Results from 2011 could not be tested, as only a few individuals were recorded then in this experiment. We could not perform these tests for the plots with different disturbance regimes separately because only a few individuals were found in undisturbed plots. We, however, tested for the effect of disturbance regime on the number of *J. montana* individuals in the cultivation experiment. We used a GLM with Poisson distribution to do this. A manipulative disturbance regime (0/1) was used as an independent variable. As the dependent variable, we used the total number of individuals in the plots of a given regime in each block sown with seed in (i) 2008, (ii) 2009 and (iii) 2010, and (iv) the sum of flowering individuals in the plots of a given regime in each block from 2008 to 2011; the habitat code was used as a covariate. All analyses were performed in R 2.14.1. (R Development Core Team 2011).

Results

The Abiot model included four of the nine abiotic parameters tested: area, slope, contact with path and presence of gravel (df error = 123, $P = 0.01$, $R^2 = 0.11$, Table 1). The Ellenberg model included four of the six indicator values: light, nutrients, temperature and continentality (df error = 123, $P < 0.001$, $R^2 = 0.17$, Table 1). The Full model included five of the 16 test variables: slope, contact with path, presence of deep soil, presence of stones and Beals index (df error = 122, $P < 0.001$, $R^2 = 0.54$, Table 1).

The Beals model identified the least and the Ellenberg model most of the habitats suitable for but unoccupied by *J. montana* (Table 2, Fig. 1). Sixty-four percent of the suitable unoccupied habitats identified by the Beals model, 43 % identified by the Abiot model and 29% identified by the Ellenberg model were also identified as suitable yet unoccupied by the Full model. AUC values largely differed among the models, the lowest being for the Abiot model (Table 2).

Table 2. – Number of suitable habitats that are not occupied by *Jasione montana*, AUC and correspondence of model predictions with the presence and number of *J. montana* individuals in the cultivation experiment in different years. R^2 are shown only for significant tests ($P < 0.05$; df error = 19); n.s. indicates non-significant tests.

Model	No. habitats	AUC	*J. montana* number				*J. montana* presence			
			2008	2009	2010	flowering 2008–2011	2008	2009	2010	flowering 2008–2011
Abiot	15	0.72	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Beals	12	0.88	n.s.	n.s.	n.s.	n.s.	**0.14**	n.s.	**0.42**	**0.46**
Ellenberg	18	0.79	n.s.	n.s.	**0.28**	**0.29**	n.s.	n.s.	n.s.	n.s.
Full	14	0.93	n.s.	n.s.	n.s.	**0.31**	**0.40**	n.s.	**0.22**	**0.32**

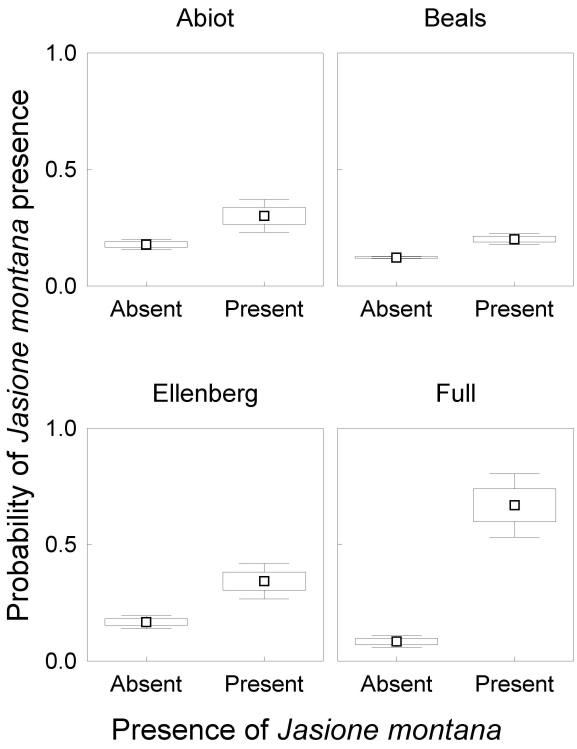

Fig. 1. – Probability of the presence of *Jasione montana* in individual habitats predicted by the models: Abiot (using abiotic parameters), Beals (Beals index values), Ellenberg (using Ellenberg indicator values) and Full (using a combination of habitat characteristics). Probabilities are shown for habitats both with and without recent presence of *J. montana* separately. Mean, SE and 1.96 SE are shown.

Jasione montana were successfully recruited in 13 of 21 habitats and survived until flowering in five habitats (including all three occupied habitats) of those sown with seed of this species. We recorded 102 vegetative individuals and no flowering individual of *J. montana* in 2008. Thirty two percent of the individuals survived from autumn 2008 to autumn 2009. We recorded 96 vegetative and one flowering individual in 2009, 29 vegetative and 15 flowering individuals in 2010 and 7 vegetative and 7 flowering individuals in 2011.

We found no significant relationship between the predictions of the Abiot model and both the presence and number of *J. montana* individuals in the cultivation experiment (Table 2). The Beals model significantly predicted the presence of *J. montana* individuals in the experiment in 2008 and 2010 and the presence of flowering individuals in this experiment (Table 2). The Ellenberg model significantly predicted the number of *J. montana* individuals in the experiment in 2010 and the number of flowering individuals in the experiment. Only the Full model significantly predicted both the presence and number of *J. montana* plants in the experiment. Specifically, the Full model significantly predicted the presence of *J. montana* individuals in 2008, in 2010 and the presence and the number of flowering individuals in the experiment (Table 2).

We also found a significant ($P < 0.05$) positive effect of disturbance on *J. montana* abundance in the cultivation experiment in 2008 (df error = 20, $P < 0.001$, $R^2 = 0.28$), 2009 (df error = 20, $P< 0.001$, $R^2 = 0.28$) and 2010 (df error = 20, $P = 0.005$, $R^2 = 0.03$, Fig. 2). Disturbance also had a significant positive effect on the total number of flowering *J. montana* individuals in the cultivation experiment (df error = 20, $P < 0.001$, $R^2 = 0.15$, Fig. 2).

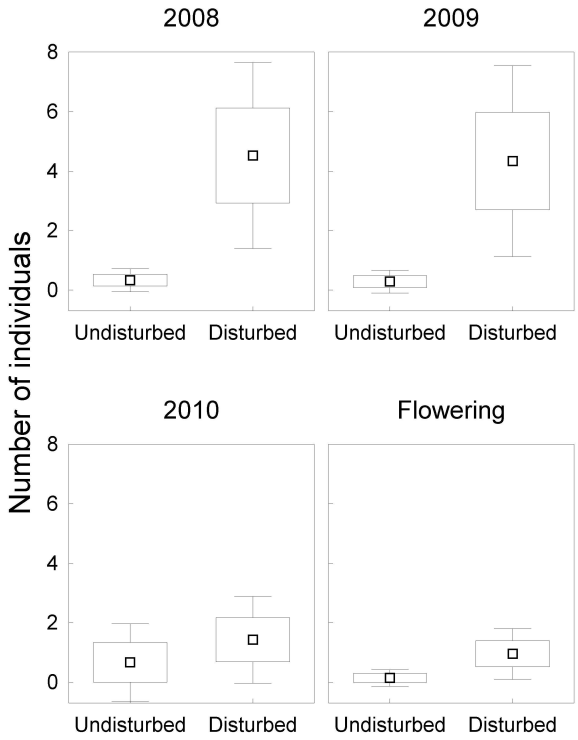

Fig. 2. – Number of individuals of *Jasione montana* in 2008, 2009 and 2010, and that of flowering individuals in 2008–2011 recorded in undisturbed and disturbed plots in the cultivation experiment. Mean, SE and 1.96 SE are shown.

Discussion

Models predicting habitat suitability

The importance of abiotic habitat characteristics for species occurrence is discussed in many previous studies (e.g., Bastin and Thomas 1999; Dupré and Ehrlén 2002; Thuiller et al. 2003). It has also been suggested that parameters derived from species composition such as Beals index or Ellenberg indicator values might be better predictors of species occurrence than abiotic habitat characteristics (e.g., Dupré and Diekmann 1998; Münzbergová 2004; Ehrlén et al. 2006; Elmendorf and Moore 2008). In this study, we found that habitat characteristics included in our models strongly influenced their predictions. Based on the classification of model performance suggested by Swets (1988), no model had a low accuracy ($0.5 < AUC < 0.7$), the models Abiot, Ellenberg and Beals were indicated as useful for some applications ($0.7 < AUC < 0.9$) and the Full model was very accurate ($AUC > 0.9$). Similarly, our comparison of the models' predictions with the results of the cultivation experiment revealed a low ability of the Abiot model to predict both the presence and abundance of *J. montana* individuals in the cultivation experiment. The Beals and Ellenberg models significantly predicted the presence and abundance of *J. montana* individuals in the cultivation experiment, respectively. Only the Full model significantly predicted both the presence and abundance of *J. montana* individuals in the cultivation experiment. This finding corroborates the results of previous studies by Tájek et al. (2011) and Hemrová et al. (2012) that identified the combination of abiotic characteristics and parameters derived from species composition as the best predictors of the presence of species in their studies. Our results indicate that it is difficult to preselect habitat characteristics that are important for species presence and demonstrate the potential advantage of parameters derived from species composition. We also found that habitat characteristics commonly used in species distribution models can prove insufficient for

predicting species distribution. By contrast, species composition can reflect not only the current habitat quality and configuration but also past or present land use, which has repeatedly been shown to be important for many species (e.g., Lindborg and Eriksson 2004; Chýlová and Münzbergová 2008; Johansson et al. 2008). Parameters derived from species composition should therefore be included among habitat characteristics commonly used in species distribution models. Their different importance for the distribution of different species has to be kept in mind, however. Of the parameters derived from species composition, we highly recommend using Beals index because of its small number of degrees of freedom along with its high explanatory power (e.g., Münzbergová 2004; Ehrlén et al. 2006; Moore and Elmendorf 2006; Hemrová et al. 2012). Beals index can be calculated from both phytosociological relevés and species lists. As recording species composition can be difficult in large areas, the possibility of using the existing databases and past records of species occurrences to compute Beals index should be considered; however, the predictive power of this approach should be estimated when applied to large areas.

Landscape dynamics of J. montana

The Full model, which was identified as the best predictor of *J. montana* presence, identified 56% of the occupancy of suitable habitats by *J. montana* in the area. A similar degree of dispersal limitation of *J. montana* was also confirmed by the cultivation experiment (60% of suitable habitats occupied). It is suggested that habitat occupancy relates to the number of available habitats in the landscape (e.g., Husband and Barrett 1996; Bastin and Thomas 1999) and also to species life-history traits (e.g., Dupré and Ehrlén 2002; Jacquemyn et al. 2003; Verheyen et al. 2004; Tremlová and Münzbergová 2007). During the course of our study, we recorded a high percentage mortality of *J. montana* in the cultivation experiment. This experiment revealed that disturbance positively affected *J. montana* recruitment and survival. Similarly, the Abiot model and the Full model that included contact of habitats with paths, i.e. parameter representing disturbance of the habitats. The importance of disturbance for species recruitment is in agreement with the findings of Parnell (1985), who observed *J. montana* in disturbed sites and of Weigelt et al. (2007) who identified *J. montana* as a poor competitor.

Reliability of models and the cultivation experiment

The Full model accurately predicted the presence of *J. montana* individuals in the landscape. This model explained more than half of the variation in *J. montana* presence in the landscape but only a third of the variation in both the abundance and presence of individuals of *J. montana* in the cultivation experiment. The discrepancy between the ability of the model to predict this species' presence in the landscape and the results of the cultivation experiment may have several explanations. Firstly, it could relate to partial suitability of the habitats, i.e., some of the plots were located in less suitable parts of the habitats. We suppose, however, that the predicted habitat suitability was reliable for the cultivation plots, since the blocks sown with seed were placed close to the phytosociological relevés and thus were representative of the habitat quality. Secondly, we could have sown an insufficient number of seeds, so the cultivation experiment consequently provided misleading results due to demographic stochasticity. This hypothesis is supported by the small number of flowering individuals recorded in this experiment (23 individuals in total in three occupied and two unoccupied habitats). We suppose, however, that *J. montana* flowered in all the suitable habitats included in this experiment because flowering *J. montana* were recorded in all the blocks sown with seed placed in habitats occupied by *J. montana*. We therefore assume that the results of the

cultivation experiment were a good indicator of habitat suitability. We also suppose, however, that the model predictions for some habitats suffered from inaccuracy in spite of their high AUC values. When habitats suitable for a species are identified, for example, for the purpose of a metapopulation study, a sensitivity analysis of the model determination of habitat suitability should be performed.

We found that long-term rather than short-term results of the cultivation experiment were significantly explained by habitat suitability. Seedling abundance could not be used as good measure of habitat unsuitability but seedling survival and their subsequent reproduction can. This finding corroborates studies suggesting that long-term rather than short-term results of sowing experiments reflect habitat suitability (e.g., Turnbull et al. 2000; Münzbergová 2004; Ehrlén et al. 2006). Finally, *J. montana* is supposed to be an annual or biennial species (Tutin 1976). We therefore expected that a short time would be needed for *J. montana* to grow into flowering individuals. Unexpectedly, most flowering individuals were recorded in the third and fourth year of the experiment. This shows that a cultivation experiment can turn out to be more time-consuming than initially thought (Ehrlén et al. 2006), even for a short-lived species.

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Appendix 1. – Correlation matrix of habitat characteristics assembled for dry acidic grassland habitats. Significant correlations ($P < 0.05$) are highlighted in **bold**.

		Area	Slope	PDSI		Contact with path	Deep soil	Shallow soil	Gravel	Stones	Beals index	Ellenberg indicator values				
				March	June							L	M	S	N	T
Slope		-0.01														
PDSI	March	0.09	**0.33**													
PDSI	June	0.05	**-0.83**	-0.09												
Contact with path		-0.15	-0.16	-0.05	0.13											
Deep soil		**-0.25**	0.10	-0.14	-0.09	-0.06										
Shallow soil		-0.04	0.04	0.01	-0.08	0.05	0.01									
Gravel		**0.22**	0.04	**0.24**	0.01	-0.07	**-0.31**	**-0.55**								
Stones		**0.35**	0.08	0.03	0.08	**-0.22**	0.16	-0.10	0.08							
Beals index		0.14	0.06	0.08	-0.05	-0.08	-0.12	0.04	0.16	0.06						
Ellenberg indicator values	L	0.02	0.13	0.11	-0.10	-0.13	0.00	0.06	0.02	0.12	**0.21**					
Ellenberg indicator values	M	-0.09	-0.12	-0.07	0.09	0.05	**0.18**	-0.08	-0.07	-0.13	**-0.28**	**-0.61**				
Ellenberg indicator values	S	-0.11	0.00	-0.09	-0.10	-0.13	**0.18**	0.09	-0.13	-0.06	**-0.27**	-0.05	**0.25**			
Ellenberg indicator values	N	-0.10	-0.09	-0.05	0.04	0.08	**0.22**	-0.10	-0.16	**-0.24**	**-0.46**	**-0.36**	**0.77**	**0.27**		
Ellenberg indicator values	T	-0.02	-0.02	0.06	0.01	0.04	-0.16	0.04	-0.02	-0.04	**-0.21**	**0.29**	**-0.33**	-0.08	-0.14	
Ellenberg indicator values	C	0.02	-0.02	-0.17	-0.05	-0.09	-0.06	0.05	-0.14	-0.15	**-0.37**	-0.07	0.09	**0.47**	**0.19**	0.05

Abbreviations: L = Light; M = Moisture; S = Soil reaction; N = Nutrients; T = Temperature; C = Continentality

Chapter 2

Colonization of central European abandoned fields by dry grassland species depends on the species richness of the source habitats: a new approach for measuring habitat isolation

Jana Knappová, Lucie Hemrová and Zuzana Münzbergová

Abstract

Abandoned fields are perceived as potential habitats for species of threatened semi-natural dry grasslands. However, information is lacking regarding how the spontaneous colonization of abandoned fields depends on the broader spatial context. We recorded the occurrence of 87 target species in 46 abandoned fields and 339 dry grasslands. We tested the effect of the isolation of abandoned fields from source grasslands on the number of dry grassland species occurring in abandoned fields either with or without habitat characteristics being used as covariates. The isolation of the fields was calculated using the distance and area (I_A) or distance and species richness (I_S) of source habitats. I_S always explained the number of grassland species in the abandoned fields better than I_A. The effect of isolation became smaller or even non-significant with the inclusion of covariates; it also changed with the method used for measuring distance (edge-to-edge or center-to-center), and it was lower when other abandoned fields were considered as additional source habitats. The different performance of the two isolation measures can be explained by the weak species–area relationship in the grasslands, indicating differences in their habitat quality. Species richness is a better proxy of habitat importance in terms of propagule source than habitat area, and the new isolation measure is therefore suitable for studying the effects of landscape structure on species richness in landscapes presenting a weak species–area relationship, such as areas exhibiting pronounced effects of land-use history. Inclusion of habitat characteristics as covariates may considerably alter conclusions regarding the effect of isolation, which might actually be overestimated when assessed separately.

Keywords: agricultural landscape, connectivity, Czech Republic, dispersal, diversity, fragmentation, *Festuco-Brometea*, secondary succession

Introduction

The extent of species-rich semi-natural grasslands has been drastically declining throughout Europe over the past century. The remaining grasslands are fragmented and scattered within the landscape and the cessation of former management practices has led to overgrowth of many localities by shrubs and trees (Lipsky 1995; Poschlod and Bonn 1998; Eriksson et al. 2002; Luoto et al. 2003; Cousins 2009a). More recently, socio-economic changes and new technologies used in agriculture have brought about the abandonment of arable fields that were no longer profitable to maintain. This could be beneficial for grassland conservation, as the direct restoration of grasslands on ex-arable land is becoming a common practice (Pywell et al. 2002; Walker et al. 2004). Nevertheless, only a limited number of former fields can be intentionally converted into grasslands, and the question arises of whether fields can be successfully colonized by grassland species, even without conservation efforts (Ruprecht 2006; Oster et al. 2009a, b).

To assess the natural colonization of abandoned fields, the number of grassland species spontaneously growing in abandoned fields compared to the pool of species present in adjacent source grasslands must be known. However, the distribution of grassland plants in abandoned fields has only rarely been studied at a landscape scale (but see Ruprecht 2006; Cousins and Aggemyr 2008 for grazed ex-fields). Rather, studies addressing spatial patterns of species richness in abandoned fields usually only compare plots at different distances from an adjacent source habitat (Cook et al. 2005; Oster et al. 2009b).

The studies examining dispersal from a single source habitat focus on species' ability to disperse over relatively short distances, and dispersal from farther source habitats is overlooked. The broader spatial context of target fields can be quantified as the isolation of a field from source grasslands. Based on theory (Hanski 1999), the species richness of a particular habitat patch is expected to decrease with increasing isolation of this patch. However, many studies in grasslands have failed to demonstrate an effect of isolation on species richness (Lindborg and Eriksson 2004; Helm et al. 2006; Lobel et al. 2006; Oster et al. 2007). This may be caused by the very slow response of perennial plants to landscape changes (Helm et al. 2006; Cousins and Eriksson 2008; Cousins 2009b), indicating that species are in fact responding to landscape structure in the past and that their distribution is not in equilibrium with the current landscape (Lindborg and Eriksson 2004; Gustavsson et al. 2007). The ongoing process of colonization of recently abandoned fields by species from current grasslands provides an excellent opportunity to assess the effect of isolation on species richness without it being obscured by historical changes in landscape structure.

Recent work has shown that plant species richness varies in response to topography-related habitat characteristics, such as site-specific solar radiation and slope (Pykala et al. 2005; Bennie et al. 2006). The species richness of grassland plants is also related to soil conditions (Janssens et al. 1998). However, studies on the effect of isolation on the species richness of grassland plants usually do not include habitat characteristics as explanatory variables in models explaining species richness (e.g., Lindborg and Eriksson 2004; Oster et al. 2007; Bruckmann et al. 2010). Therefore, the resulting effect of habitat isolation on species richness might be in fact overestimated due to spatial correlation of habitat characteristics and isolation. We aim to compare the effect of isolation of abandoned fields from source grasslands with and without inclusion of habitat characteristics.

To describe habitat isolation, different studies use very different measures (Moilanen and Nieminen 2002; Kindlmann and Burel 2008; Prugh 2009). However, all measures that have been used thus far to study the effect of habitat isolation on species richness are based on the distance from possible sources and/or the size of the source habitat and do not consider the species richness at the sources. The use of area in assessing isolation is based on the assumption that larger patches exhibit higher species richness and host larger populations and, thus, may provide more possible colonizers for the target patch (Hanski

1999; Kiviniemi 2008). In fragmented grasslands, however, a number of studies have failed to reveal a positive relationship between patch area and species richness (Eriksson et al. 1995; Kiviniemi and Eriksson 1999; Partel and Zobel 1999) or patch area and population size (Eriksson and Ehrlen 2001; Bruun 2005). This may be caused by different habitat conditions of the source patches resulting from factors such as different land-use histories at these patches (e.g., Cousins 2001; Lindborg et al. 2005; Chylova and Munzbergova 2008). We therefore hypothesize that using the species richness of surrounding source habitats instead of area may provide more meaningful results than when calculating isolation based on area.

Our study area in northern Bohemia, Czech Republic is situated in a landscape associated with a long tradition of agriculture and contains both grassland fragments and abandoned fields. This provides an excellent opportunity to examine and separate factors limiting the richness of grassland plants in abandoned fields. In this study, we performed an extensive field survey asking the following questions: (i) How many dry grassland species were able to colonize arable fields abandoned in last two decades? (ii) What is the effect of isolation of an abandoned field from source dry grasslands on number of grassland species colonizing the field? (iii) How does the detected effect of isolation change with the method used for its calculation? (iv) How does the detected effect of isolation change when habitat characteristics are included as covariates?

To answer these questions, we recorded the occurrence of 87 dry grassland plant species in 339 source dry grasslands and 46 target abandoned fields. For each abandoned field, we assessed a number of habitat characteristics that could be used as covariates. Finally, two different measures of the isolation of each field were calculated based either on area and distance or on species richness and the distance of surrounding source grasslands.

Methods

Study area and target species

The study area (8.5 by 8.5 km) is situated in the northern part of the Czech Republic (boundaries: 50°33′19.3″N, 14°14′25.1″E–50°33′47.4″N, 14°21′36.2″E; 50°28′46.3″N, 14°15′5.2″E–50°29′47.2″N, 14°21′40.9″E) at 200–270 m a.s.l. The long-term average temperature in the region is 7.7°C, and the long-term normal precipitation is 612 mm (Web 1). The prevailing bedrocks consist of sediments of different ages and origins, mainly sandstones and loess loams. The area is associated with a long tradition of agriculture with a prevalence crop production, accompanied by vineyards on steeper southern slopes and hop fields in alluvial areas. At present, agricultural land covers more than 70% of the study area (see map in Appendix 1). Natural vegetation is represented by remnants of oak-hornbeam and thermophilous oak forests (alliance *Carpinion* and *Quercion petrae*, Ellenberg 1988), with total cover of approximately 10% within the study area. Calcareous dry grasslands (alliance *Bromion erecti*, Ellenberg 1988) occur in small fragments totaling 4% of the study area, and they host a vast small-scale diversity of vascular plants (Munzbergova 2004, Chylova and Munzbergova 2008), including a number of threatened species. At present, most of the grasslands in the region are not managed, and occasionally, some of them are completely destroyed by human activities (e.g., plowing or development of solar power plants). A previous study in the same region demonstrated some portions of the current area of dry grasslands were arable fields in the 1950s or even in 1980s (Chylova and Munzbergova 2008), suggesting that grassland species have been able to spread into novel habitats. In the past, fields, orchards, pastures, vineyards and grasslands formed a small-grain heterogeneous mosaic. Therefore, a mixture of different land use

histories can be found containing both continuous grasslands and relatively recently (e.g., in the 1980s) abandoned fields within a single current grassland (Chylova and Munzbergova 2008). However, due to changes in agriculture, the fields in the current landscape are much larger and farther from the source grasslands than they were in the past. Moreover, increased application of fertilizers and the use of deep cultivation in the last decades might have considerably altered soil conditions. We therefore assume that species' colonization of currently abandoned fields will be limited both by habitat suitability and species' dispersal ability. Fields abandoned in last 20 years are already overgrown with grasses and ruderal herbaceous vegetation, e.g., *Arrhenatherum elatius*, *Dactylis glomerata*, *Cirsium arvense*, *Melilotus albus* and *M. officinalis*, and they make up approximately 1% of the study area.

Data collection

Field data collection

Based on studies performed within the same region (Munzbergova 2004; Tremlova and Munzbergova 2007; Chylova and Munzbergova 2008), we selected 87 target species as species restricted to dry grassland fragments (Table 1).

Table 1. – List of target dry grassland species. For each species, the percentages of occupied (AF) abandoned fields (from total 46) and (DG) dry grasslands (from total 339) are shown.

Target species	Percentage occupied		Target species	Percentage occupied	
	AF	DG		AF	DG
Agrimonia eupatoria	96	92	*Koeleria pyramidata*	7	19
Coronilla varia	93	89	*Tanacetum corymbosum*	7	17
Fragaria viridis	89	81	*Asperula cynanchica*	7	15
Origanum vulgare	78	49	*Dianthus carthusianorum*	7	14
Inula salicina	76	67	*Veronica austriaca* subsp. *teucrium*	4	13
Festuca rupicola	74	75	*Aster amellus*	4	9
Knautia arvensis	72	88	*Melampyrum arvense*	4	9
Centaurea jacea	72	70	*Inula hirta*	4	3
Astragalus glycyphyllos	72	59	*Artemisia campestris*	4	2
Euphorbia cyparissias	67	81	*Centaurea rhenana*	4	2
Galium verum	67	73	*Thymus praecox*	2	27
Bupleurum falcatum	65	65	*Anthyllis vulneraria*	2	22
Brachypodium pinnatum	59	84	*Peucedanum cervaria*	2	16
Lotus corniculatus	57	78	*Genista tinctoria*	2	12
Salvia verticillata	54	50	*Potentilla arenaria*	2	11
Astragalus cicer	52	35	*Teucrium chamaedrys*	0	23
Centaurea scabiosa	48	65	*Helianthemum nummularium*	0	20
Trifolium medium	48	65	*subsp. grandiflorum*		
Scabiosa ochroleuca	48	54	*Trifolium montanum*	0	18
Stachys recta	41	36	*Carex humilis*	0	17
Plantago media	39	65	*Anemone sylvestris*	0	15
Linum catharticum	37	66	*Polygala vulgaris*	0	15
Sanguisorba minor	30	71	*Geranium sanguineum*	0	10
Carlina vulgaris	28	25	*Asperula tinctoria*	0	9
Eryngium campestre	26	46	*Anthericum ramosum*	0	8
Bromus erectus	22	40	*Melampyrum nemorosum*	0	7
Gentiana cruciata	22	17	*Sesleria albicans*	0	7
Cirsium eriophorum	22	9	*Aster linosyris*	0	5

Table 1 continued

Target species	Percentage occupied		Target species	Percentage occupied	
	AF	DG		AF	DG
Hieracium pilosella	20	25	*Filipendula vulgaris*	0	5
Pimpinella saxifraga	17	48	*Globularia elongata*	0	5
Medicago falcata	11	37	*Linum tenuifolium*	0	5
Primula veris	11	31	*Campanula rotundifolia*	0	4
Ononis spinosa	11	22	*Listera ovata*	0	4
Euphrasia rostkoviana	11	3	*Linum flavum*	0	3
Leontodon hispidus	9	46	*Onobrychis viciifolia*	0	3
Briza media	9	44	*Scorzonera hispanica*	0	2
Potentilla heptaphylla	9	42	*Seseli hippomarathrum*	0	2
Carex flacca	9	40	*Thesium linophyllon*	0	2
Carex tomentosa	9	22	*Campanula glomerata*	0	1
Salvia nemorosa	9	13	*Coronilla vaginalis*	0	1
Salvia pratensis	7	68	*Gymnadenia conopsea*	0	1
Prunella vulgaris	7	32	*Laserpitium latifolium*	0	1
Cirsium acaule	7	29	*Melampyrum cristatum*	0	1
Prunella grandiflora	7	29	*Scabiosa canescens*	0	1

The nomenclature follows Tutin et al. (1964–1983).

In 2009, using GPS, we located all fields abandoned in the last 15 years and all source grasslands within the study area. A total of 46 abandoned fields ranging in size from 815 to 50,222 m^2 and 339 dry grasslands ranging in size from 6 to 274,800 m^2 were included. We defined a dry grassland as a site with visually homogenous vegetation separated from other localities by an unsuitable area and hosting at least one of the target dry grassland species. In cases of abrupt vegetation change within continuous grassland, the parts with different vegetation were treated as different localities. These cases were not common; in all of them there was a visual topographic barrier between the localities such as a small ditch or change of slope from very steep to flat.

In all of the abandoned fields and dry grasslands, we recorded the occurrence of each of 87 target species. During the field survey, we recorded only adult, usually flowering, individuals because small juvenile plants and seedlings are almost impossible to detect in the dense vegetation of abandoned fields. For the purpose of our study, the species found in the abandoned fields are referred to as *generalists*. Species only found in the dry grasslands are referred to as *specialists*. We use these terms for simplicity, mainly to separate the two groups of species, which could be also classified as *early* and *late colonizers* or *good* and *poor colonizers*.

Data on habitat characteristics

For each abandoned field, we determined several types of habitat characteristics (Table 2). Information on the time since the abandonment of each field (further referred to as "Age") was obtained through personal communication with landowners. As this information was only approximate, we divided the fields into three age categories: up to 7, 10 or 15 years. Several fields were also seeded with a commercial seed mixture in the last year before abandonment. The commercial seed mixture consisted of a few productive grasses and legumes (*Dactylis glomerata, Festuca pratensis, Lolium perenne, Lolium multiflorum* and *Trifolium pratense*) and did not contain any of the target species. Information related to seeding is important because it may influence the establishment success of the target species at the study sites.

Table 2. – List of parameters collected for each abandoned field (N = 46) and their effect on the species richness of the abandoned fields. Parameters in **bold** were selected by step-wise regression and, thus, were included in the final model. The sign ± indicates a positive/negative relationship of the parameters in the model.

Parameter	Df	Type		R^2
Coordinates				0.15
x	**1**	**continual**		
y	**1**	**continual**		
x*y	**1**	**continual**		
Habitat characteristics				0.48
Age	1	continual		
Seeding	**1**	**binomial**	**-**	**0.05**
Area	**1**	**log(continual)**	**+**	**0.05**
TWI	1	continual		
Slope	**1**	**continual**	**+**	**0.12**
PDSI_December	1	continual		
PDSI_January	1	continual		
PDSI_February	1	continual		
PDSI_March	**1**	**continual**	**-**	**<0.01**
PDSI_April	1	continual		
PDSI_May	**1**	**continual**	**+**	**0.01**
PDSI_June	**1**	**continual**	**-**	**0.17**
Geology	**7**	**factorial**		**0.08**

TWI topographic wetness index; *PDSI* potential direct solar irradiation

We used ArcGIS 9.2 (ESRI 2006) to obtain mean values for the TWI (topographic wetness index), Slope and potential direct solar irradiation (PDSI) from December to June for each abandoned field. Based on digital geological data, we also assessed the percentage cover of eight bedrock types in each abandoned field (further referred to as Geology, Table 2). See Appendix 2 for technical details of the GIS analyses and an extended description of habitat characteristics.

Isolation of abandoned fields

The majority of studies assessing the effect of isolation on plant species richness in grasslands use the isolation measure originating from the Incidence Function Model of metapopulation dynamics (Hanski 1994). To incorporate different distances of source patches, this measure uses a negative exponential dispersal kernel (the probability density function of the dispersal distance for an individual or population), with parameter α scaling the effect of distance to migration. However, parameter α is species specific and is difficult to accurately estimate in studies on multiple species. We therefore decided to use a simpler quadratic rational dispersal kernel (e.g., Tremlova and Munzbergova 2007).

First, we calculated the isolation of each abandoned field (I_A) as the mass of the surrounding source dry grasslands weighted by their distance to the target abandoned field following Eq. 1.

$$I_{Aj} = -\log \sum_{k=1}^{n} \left[(A_k / d_{jk}^2) \right], \; j \neq k$$

where I_j is the isolation of abandoned field j; k represents all of the surrounding grasslands within a 0.5 km distance of abandoned field j; A_k is the area of grassland k; and d_{jk} is the distance between abandoned field j and grassland k.

Second, we replaced the area of the surrounding grasslands with the number of target species occurring in each grassland and calculated isolation (I_S) following Eq. 2.

$$I_{Sj} = -\log \sum_{k=1}^{n} \left[(S_k / d_{jk}^2) \right], \; j \neq k$$

where I_j is the isolation of abandoned field j; k represents all of the surrounding grasslands within a 0.5 km distance of abandoned field j; S_k is the number of target species in grassland k; and d_{jk} is the distance between abandoned field j and grassland k. We measured d_{jk} either as the center-to-center or edge-to-edge (shortest) distance.

The other surrounding fields could theoretically also serve as propagule sources for target species. Therefore, we considered surrounding abandoned fields as additional source habitats when calculating isolation and compared the results obtained from the models when abandoned fields were not considered.

The mean edge-to-edge distances between the source and target habitats were 253 and 226.4 m (range 1–500 m) when source habitats were represented either by grasslands or both grasslands and abandoned fields, respectively. The mean center-to-center distances were 288.9 and 271.5 m (range 20–500 m), respectively.

The resulting isolation value was higher for more isolated sites, i.e., abandoned fields that were farther from source grasslands and/or when the source grasslands were smaller (I_A) or hosted fewer target species (I_S). For the most isolated abandoned fields, I_A = 1.88, and I_S = 3.83. For the least isolated abandoned fields, I_A = -5.12, and I_S = -2.01.

Data analysis

To assess differences in the number of target species occurring in individual grasslands and abandoned fields, differences in the areas of the two habitat types must be taken into account. In a linear regression, the number of target species was employed as a dependent variable, and habitat type (grassland/field) and the logarithm of area and their interaction were used as independent variables. The relationship between the number of target species and the logarithm of habitat area was also analyzed separately for the two habitat types using linear regression. In grasslands, it was further tested separately for specialist and generalist species. Because some of the grasslands were either larger than the largest abandoned field or smaller than the smallest abandoned field, we also tested the species–area relationship using only grasslands within the same span of areas as the abandoned fields.

To identify factors determining the number of species occurring in the abandoned fields, we used linear regression. First, we corrected for possible spatial gradients within the study area (such as related to climate). We used the x and y coordinates of the centers of abandoned fields and their interaction (x*y) as independent variables (Table 2) and tested their effects on the number of target species in the abandoned fields. The significant coordinates were thus used as covariates in selecting habitat characteristics. Some habitat characteristics were strongly correlated with each other (Appendix 3). Therefore, we selected only the most important ones by bi-directional step-wise selection based on the AIC (Akaike Information Criterion, a formof penalized log-likelihood analysis, Crawley 2002).

Finally, we tested the overall effect of isolation of the abandoned fields, I_A (i.e., calculated based on the area of surrounding grasslands) or I_S (i.e., calculated based on the species richness of surrounding grasslands), on the number of target species occurring in the abandoned fields. To test the pure effect of isolation, pre-selected coordinates and habitat characteristics were used as covariates. All of the analyses were performed in S-Plus 2000 (MathSoft 1999).

Results

We found 59 out of the 87 (i.e., 68%) target dry grassland species in the abandoned fields. Twenty-eight species were not found in the abandoned fields and were therefore considered to be grassland specialists (Table 1).

The number of target species in each abandoned field varied from 3 to 34 (mean 18), whereas the number of target species in the dry grasslands varied from 5 to 63 (mean 26). When considering only generalists (species occurring in at least one abandoned field), the number of target species occurring in the dry grasslands varied from 3 to 47 (mean 24). Grasslands harbored significantly more target species than abandoned fields when considering both generalists and specialists ($F_{1,382}$ = 55.88, P < 0.001, R^2 = 0.12) or generalists alone ($F_{1,382}$ = 47.66, P < 0.001, R^2 = 0.10; Fig. 1).

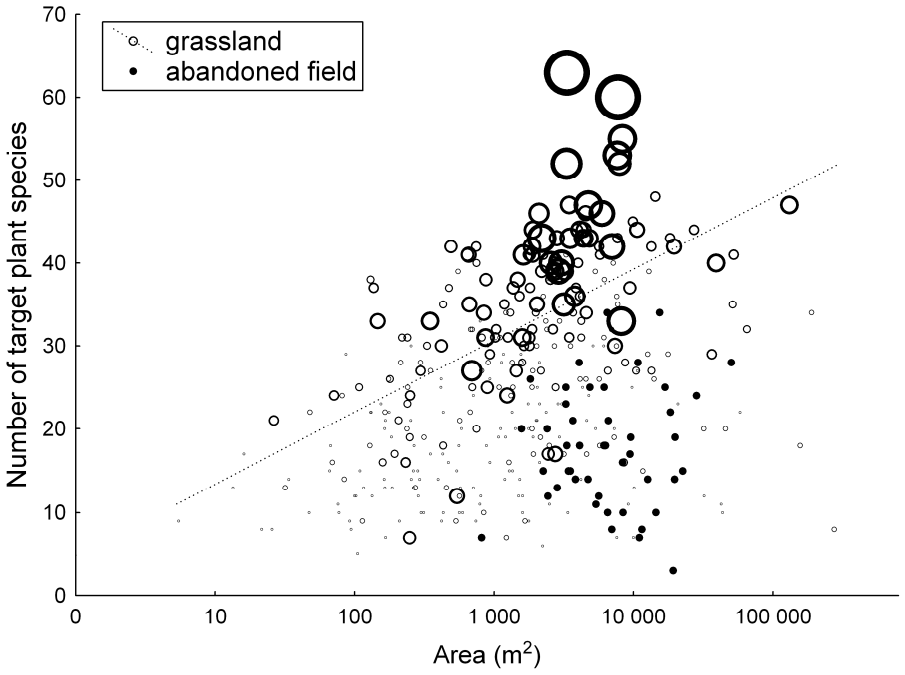

Fig. 1. – Relationship between habitat area and the number of target species in grasslands and in abandoned fields. The size of the *open circles* denotes the number of grassland specialists (species not found in abandoned fields). Grasslands with no specialists are depicted as *small dots*. A *regression line* is shown for the full range of grasslands ($F_{1,337}$ = 68.85, R^2 = 0.170, P < 0.001); the species–area relationship was not significant for abandoned fields ($F_{1,44}$ = 0.047, P = 0.829) or for grasslands when using the same span of areas as for abandoned fields ($F_{1,206}$ = 3.12, P = 0.078).

A significantly positive relationship between the number of target species in dry grasslands and the logarithm of grassland area was found only when analyzing the whole range of grasslands (Fig. 1; $F_{1,337}$ = 68.85, R^2 = 0.170, P < 0.001 for all target species; $F_{1,337}$ = 70.320, R^2 = 0.173, P < 0.001 for generalists; $F_{1,337}$ = 22.840, R^2 = 0.063, P < 0.001 for specialists) However, the species–area relationships were relatively weak, indicating differences in habitat quality between individual grasslands. The weakest species–area relationship was found when it was calculated only for specialists. However, when we considered only grasslands within the same span of areas as the abandoned fields, the species–area relationship was not significant ($F_{1,206}$ = 3.12, P = 0.078 for all species; $F_{1,206}$ = 3.53, P = 0.06 for generalists; $F_{1,206}$ = 0.66, P = 0.419 for specialists), nor was the relationship between the number of target species occurring in abandoned fields and field area ($F_{1,44}$ = 0.047, P = 0.829).

All of the three tested spatial coordinates (x, y and x*y) and seven out of 13 habitat characteristics were selected in a stepwise analysis in the most parsimonious model explaining the number of target species in abandoned fields (Table 2). We found a negative effect of seeding and PDSI in March and June and positive effects of area, slope and PDSI in May (Table 2). The topographic wetness index (TWI) and field age were not included in final model.

More target species always occurred in less isolated abandoned fields. When habitat characteristics were used as covariates, the number of target species in abandoned fields was significantly affected by I_A (calculated using the area of source habitats) and I_S (calculated using the species richness of target species on source habitats) only when edge-to-edge distance and grasslands alone as source habitats were used for the calculations (Table 3). Isolation I_S explained 34% more variability in the number of target species in abandoned fields than isolation I_A. In contrast, when habitat characteristics were not included, isolation I_S always had a significant effect on the number of target species in abandoned fields, and the effect of I_A was never significant (Table 3). The effect of isolation was generally more pronounced when only grasslands were used as source habitats. The explained variability also differed between models using different measures of distance (edge-to-edge and center-to-center; Table 3).

Table 3. – Variability explained by the two measures of isolation in different models. I_A was based on the area of source habitats, and I_S was based on the number of target species in the source habitats. Habitat characteristics were selected in step-wise regression (**Table 2**). Values in **bold** are significant on $P < 0.05$.

Habitat characteristics	Distance	Source habitats	I_A			I_S		
			R^2	F	*P*	R^2	F	*P*
Included	Edge-to-edge	DG	**0.05**	**4.38**	**0.046**	**0.07**	**6.19**	**0.019**
		DG + AF	-	0.47	0.500	-	1.24	0.275
	Center-to-center	DG	-	0.56	0.460	-	2.55	0.122
		DG + AF	-	0.19	0.663	-	0.12	0.732
Not included	Edge-to-edge	DG	-	1.76	0.191	**0.10**	**4.82**	**0.033**
		DG + AF	-	1.18	0.284	**0.10**	**4.95**	**0.031**
	Center-to-center	DG	-	1.90	0.175	**0.16**	**8.54**	**0.005**
		DG + AF	-	1.14	0.291	**0.12**	**6.16**	**0.017**

DG Dry grasslands; *AF* Abandoned fields

Discussion

Habitat isolation is known to be an important factor reducing the species richness of dispersal-limited taxa in a wide range of habitats. For grassland plants, however, the effect of present habitat isolation on species richness is often thought to be obscured by land use history (Lindborg and Eriksson 2004; Helm et al. 2006; Gustavsson et al. 2007). Even highly isolated grasslands might be species rich due to the longevity and persistence of species from a time when the grassland was larger and/or more connected to other grasslands. Here, we studied the colonization patterns of grassland species in recently abandoned fields, and the distribution of dry grassland species in these habitats should only reflect the present landscape structure. We showed that the effect of isolation strongly depends on the inclusion of habitat characteristics in the model as well as on the parameters used when calculating isolation.

The new approach developed in the present study for measuring isolation based on the species richness of source habitats (I_S) always produced better results than a commonly used area-based measure (I_A). This confirmed our hypothesis that the area of surrounding

grasslands may not fully reflect their importance as a source of propagules. The use of area-based isolation (I_A) is justified by the fact that larger habitats are generally richer in species and host larger populations. However, in many previous studies in grasslands (e.g., Eriksson and Ehrlen 2001; Lindborg and Eriksson 2004; Bruun 2005; Helm et al. 2006; Lobel et al. 2006), species–area or population size–area relationships were found to be weak or even non-existent. Similarly, our results revealed that the most species-rich grasslands were not the largest by far (Fig. 1), suggesting that factors other than area are more important for the species richness of the grasslands in our study system.

To deal with differences among propagule sources, Ruprecht (2006) weighted the areas of source grasslands according to their phytosociological classification. However, this type of approach relies on division of habitats into several categories and may not be optimal. We suggest that species richness might be a better proxy of habitat importance in terms of propagule sources than vegetation type, and our new measure of isolation based on the species richness of source habitats (I_S) can be more widely applied.

It is likely that isolation could be interconnected with important habitat characteristics. Where conditions are more suitable for grassland species, grasslands might be more abundant and clumped and therefore less isolated compare to areas with less suitable conditions. Indeed, when habitat characteristics were included, Lobel et al. (2006) did not demonstrate an effect of the isolation of grassland fragments on plant species richness, and Adriaens et al. (2006) found only a marginal effect of habitat isolation on a few functional of groups of plant species. Other studies demonstrating the effect of habitat isolation on the species richness in grasslands have not taken habitat characteristics into account (e.g., Reitalu et al. 2009; Bruckmann et al. 2010). Our results confirm that inclusion of habitat characteristics as covariates might considerably alter conclusions regarding the effect of isolation.

The relative importance of isolation also changed when different measurements of distance were applied. When calculating the isolation of individual abandoned fields, we considered all source habitats within a distance of less than 0.5 km. As a consequence, when using edge-to-edge distance, we included 3–4 more source habitats on average than when using center-to-center distance. Therefore, isolation calculated using edge-to-edge distance was systematically lower than isolation based on center-to-center distance. The greater amount of variability explained by isolation based on center-to-center than on edge-to-edge distance suggests that grassland plants are limited by dispersal at distances even shorter than 0.5 km. Nevertheless, the above-mentioned pattern only held when habitat characteristics were not included in the model. In contrast, isolation based on edge-to-edge distance was the only significant isolation measure when covariates were included. Edge-to-edge isolation measures isolation at a larger spatial scale, and it may therefore not be as strongly affected by the habitat conditions of the sites as is the center-to-center measure of isolation.

The populations of target species found in abandoned fields typically consisted of a few individuals to tens of individuals, and their long-term prospects are therefore uncertain. Nevertheless, even transient populations might positively influence species persistence at the landscape scale (Loehle 2007). Our results thus confirm the importance of abandoned fields in the landscape dynamics of dry grassland species. Two-thirds of the target species were able to become established and could potentially spread farther in the landscape. However, when we used abandoned fields as additional source habitats for calculating isolation, the resulting effect of isolation was almost always less pronounced than when using only grasslands as source habitats. The importance of abandoned fields as sources of propagules of dry grassland species is therefore rather low in the study area.

There is increasing evidence that different processes control the species richness as well as the distributions of specialist and generalist species (Pandit et al. 2009) and that these two groups of species respond differently to landscape changes (With and Crist

1995). Bartha et al. (2003) showed that the influx of new colonizers was highest in the first 5–6 years after field abandonment, with much lower numbers of new species appearing later. The fields surveyed in our study were abandoned a maximum of 15 years ago and we can therefore expect that most of the species with the ability to colonize the abandoned fields would have already done so. Therefore, our definition of grassland specialists (species that were not able to colonize any of the abandoned fields in the time span of 15 years) to some degree reflects poor dispersal abilities of species together with narrow habitat requirements.

The difference in the strength of the species–area relationship between specialists and generalists confirms that there is an obvious difference between the landscape dynamics of the two groups and that our division is not arbitrary. Moreover, relaxed species–area relationship of specialists in grasslands implies that habitat quality is more important for their distribution than habitat area. Habitat quality might be associated with certain specific habitat conditions, either abiotic (such as pH or water holding capacity; Munzbergova 2004; Lobel et al. 2006) or biotic (such as character of soil biota, Hartnett and Wilson 1999; van der Heijden et al. 2008). Both biotic and abiotic conditions are likely to be influenced by the land-use history of a site (e.g., Karlik and Poschlod 2009; Oehl et al. 2010; Postma-Blaauw et al. 2010). We therefore suggest that similar to "ancient forest species" (Honnay et al. 1998; Hermy et al. 1999), our specialists are restricted to grasslands with long continuity of land-use. However, this needs to be further tested. Our results nevertheless show that pure presence-absence surveys carried out in grasslands and fields may help to distinguish the most specialized species, which are likely to suffer most from the loss of their habitat.

In our extensive field survey, two-thirds of the target dry grassland species were found in fields abandoned in the last two decades. However, the number of target species in abandoned fields was still significantly lower than the number of target species found in grasslands. Moreover, the difference was not due to specialists missing in the abandoned fields, it holds also when compared only numbers of generalist species on fields and grasslands. This implies that even for those species that can reach abandoned fields, some limitations still exist.

The suitability of abandoned fields for dry grassland species may be decreased by numerous factors. Because the simple species–area relationship was not significant for the abandoned fields, we assume that grassland plants do not benefit from the greater heterogeneity of larger fields because they are only able to colonize a few, more open sites. The extent of these suitable sites within each field is influenced more by other habitat characteristics than by field area. We assume the strongest negative effect to be associated with vigorously growing grass and weedy species. Indeed, fewer target species were found in fields that were seeded with a commercial seed mixture prior abandonment resulting in the formation of dense vegetation cover. Similarly, in steeper fields, nutrients are washed away faster than in flat fields, and vegetation becomes less dense and more hospitable for new colonizers.

Conclusions

We revealed an unexpectedly high success of grassland species in the colonization of fields abandoned in the last two decades. These abandoned fields may thus play an important role in the landscape dynamics of many grassland species, but their dispersal is probably often limited to short distances (less than 0.5 km). The proximity of species-rich rather than large source habitats was shown to be important for field colonization. Our new isolation measure using the distance and species richness of surrounding habitats may be helpful in studying the effects of landscape structure on species richness in landscapes with

pronounced effects of land-use history or other important factors reducing the species–area relationship.

Assessing the species richness of source habitats would appear to be much more time-consuming than just calculating their areas. However, in the majority of studies addressing habitat isolation, there is no distinction made between target and source habitats, and species richness is known for all habitats under study to be the main response variable. Therefore, there is no additional effort needed for this type of assessment (except for substitution into a formula), and our approach might be easily applied and further tested.

We have also shown that inclusion of habitat characteristics as covariates may considerably alter conclusions about the effects of isolation, which could actually be overestimated when assessed separately. We therefore highly recommend considering habitat characteristics when studying habitat isolation. Through comparison of results obtained with and without inclusion of habitat characteristics, we may gain novel insights into factors affecting species richness.

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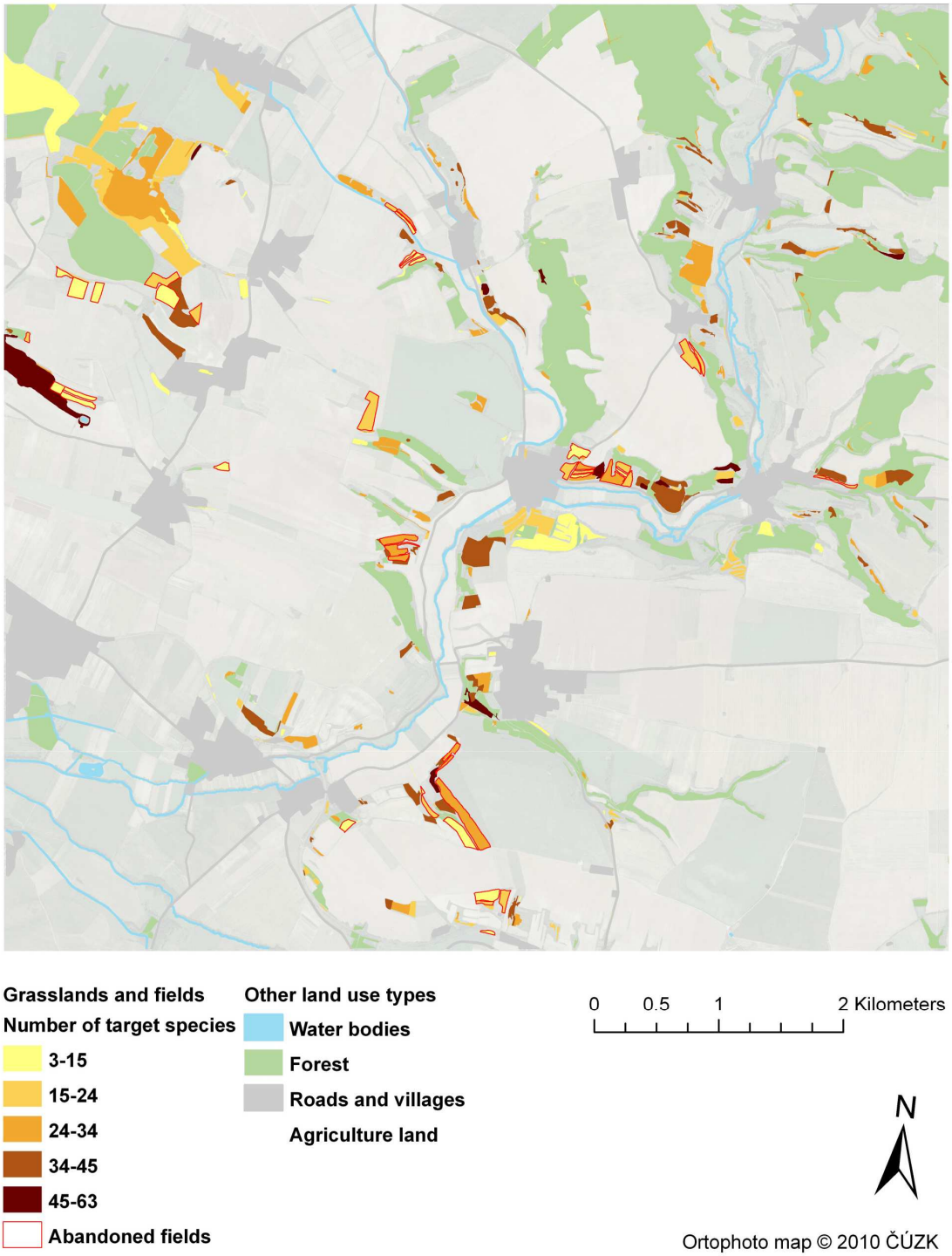
Appendix 1. – Map of study area.



Appendix 2. – Detailed information on the calculation of habitat characteristics

For the whole study area, we constructed a digital elevation model (DEM) with a 10 m grid size based on digital contour lines (1:10 000, 2 m vertical distance) provided by the Czech Office for Surveying, Mapping and Cadastre. As a gridding technique, we used ANUDEM (Hutchinson 1989) implemented in ArcGIS 9.2 (ESRI 2006). Based on the DEM, we created grids of slopes and potential direct solar irradiation (PDSI) for the 21st day of December to June using ArcGIS 9.2 (ESRI 2006).

We also calculated the topographic wetness index (TWI) using SAGA (Web 2) based on the slope grid and specific catchment area grid as TWI = ln (As/tan b), where As is the specific catchment area (the cumulative upslope area draining through a cell divided by the contour width), and b is the local slope (Beven and Kirkby 1979). As suggested by the critical study performed by Kopecky and Cizkova (2010), we used the method of Zevenbergen and Thorne (1987) to create the slope grid and the multiple flow routing algorithms of Quinn et al. (1991) to create the specific catchment area grid.

For each abandoned field, we calculated mean values of the slope, TWI and PDSI for December through June. The slope varied between 3-17° with a median of 8.5°. TWI is a non-dimensional index used to describe spatial soil moisture patterns. When simplified, higher TWI values denote higher soil moisture. In our study, TWI reached values between 7.7-14.5 with a median of 9.4. PDSI sums up the radiation per square meter at a particular site throughout day. In the study area it is lowest in December, when it varies between 121 and 274 kw.m-2.day-1 (median 417 kw.m-2.day-1), and reaches maximum in June, when it varies between 5205 and 5791 kw.m-2.day-1 (median 5702 kw.m-2.day-1).

We also used digital geological data for bedrock (GEOCR 25, 1:25 000 provided by the Czech Geological Survey) to assess the relative extent of each bedrock type in each abandoned field. Eight bedrock type categories occurred in the area: sandstone, sediment, loess + loess loam, sand, loam, gravel, claystone + marlite + siltstone and limestone. For each field, the percentage of each bedrock type was assessed, summing to 1.

Appendix 3. – Correlation matrix parameters assembled for abandoned fields. Significant correlations ($P < 0.05$) are highlighted in **bold**.

	Coordinates			Age	Seed	Area	TWI	Slope	PDSI							SanSt	Sedi	LoLo	Clay	Sand	Grav	CMSi	LimSt
	x	y	x*y						Dec	Jan	Feb	Mar	Apr	May	Jun								
y	-0.28																						
x*y	**0.71**	**0.46**																					
Age	**0.46**	-0.04	**0.41**																				
Seed	**0.37**	0.16	**0.47**	**0.51**																			
Area	-0.20	0.01	-0.18	-0.09	0.07																		
TWI	-0.23	-0.22	**-0.36**	0.02	-0.18	-0.12																	
Slope	**0.38**	**0.31**	**0.55**	0.09	0.23	-0.28	**-0.67**																
Dec	0.04	**0.36**	0.25	-0.06	0.24	-0.08	**-0.50**	**0.44**															
Jan	0.05	**0.38**	0.26	-0.07	0.23	-0.09	**-0.48**	**0.43**	**1.00**														
Feb	0.05	**0.38**	0.26	-0.07	0.23	-0.09	**-0.47**	**0.42**	**1.00**	**1.00**													
Mar	0.03	**0.38**	0.24	-0.08	0.23	-0.08	**-0.45**	**0.39**	**1.00**	**1.00**	**1.00**												
Apr	-0.04	**0.38**	0.18	-0.10	0.22	-0.03	**-0.41**	**0.30**	**0.98**	**0.99**	**0.99**	**0.99**											
May	-0.09	**0.37**	0.13	-0.11	0.21	0.01	**-0.38**	0.22	**0.97**	**0.97**	**0.97**	**0.98**	**1.00**										
Jun	-0.15	**0.37**	0.07	-0.13	0.20	0.06	**-0.34**	0.14	**0.94**	**0.94**	**0.95**	**0.96**	**0.98**	**1.00**									
SanSt	**0.63**	**-0.43**	0.29	0.16	**0.35**	0.07	-0.20	0.02	0.06	0.05	0.05	0.04	0.03	0.02	0.01								
Sedi	0.13	0.06	0.15	-0.14	-0.21	-0.16	0.27	-0.08	-0.12	-0.07	-0.06	-0.06	-0.07	-0.08	-0.08	-0.15							
LoLo	0.06	0.24	0.24	-0.07	-0.03	-0.01	-0.09	-0.02	0.04	0.04	0.05	0.05	0.05	0.05	0.05	-0.22	0.07						
Clay	**-0.35**	0.14	-0.21	0.06	-0.20	-0.01	-0.04	0.15	-0.06	-0.07	-0.08	-0.08	-0.09	-0.09	-0.09	**-0.64**	-0.21	-0.17					
Sand	-0.15	-0.04	-0.14	**0.30**	-0.09	-0.20	0.09	0.13	-0.19	-0.20	-0.20	-0.21	-0.24	-0.26	-0.28	**-0.51**	-0.18	-0.14	**0.74**				
Grav	**-0.35**	0.14	-0.21	0.06	-0.20	-0.01	-0.04	0.15	-0.06	-0.07	-0.08	-0.08	-0.09	-0.09	-0.09	**-0.64**	-0.21	-0.17	**1.00**	**0.74**			
CMSi	**-0.44**	0.26	-0.27	-0.14	-0.18	0.12	0.26	-0.23	0.09	0.08	0.09	0.10	0.13	0.16	0.19	-0.28	-0.09	-0.09	-0.15	-0.16	-0.15		
LimSt	**-0.51**	0.22	**-0.32**	**-0.30**	-0.02	-0.02	-0.01	0.02	0.05	0.05	0.05	0.05	0.07	0.09	0.10	**-0.33**	-0.11	-0.10	0.04	-0.09	0.04	-0.02	
I_A	-0.20	0.00	-0.21	-0.14	0.01	0.26	**-0.31**	0.24	0.20	0.19	0.18	0.18	0.18	0.18	0.18	-0.18	-0.21	-0.11	**0.35**	0.12	**0.35**	0.06	0.05
I_S	-0.06	-0.13	-0.17	-0.09	-0.02	0.26	-0.27	0.17	0.10	0.09	0.09	0.09	0.08	0.08	0.08	-0.03	-0.21	-0.10	0.23	0.07	0.23	0.08	-0.12

Abbreviations: Seed = Seeding; Dec = December; Jan = January; Feb = February; Mar = March; Apr = April; Jun = June; SanSt = Sandstone; Sedi = Sediment; LoLo = Loess + loess loam; Grav = Gravel CMSi = Clay stone + marlite + siltstone; LimSt = Lim

Chapter 3

Effects of plant traits on species response to present and historical habitat configuration

Lucie Hemrová and Zuzana Münzbergová

Abstract

Habitat configuration is considered to be an important factor affecting distribution of plant species. Although a number of studies identify plant life-history traits responsible for species distribution in fragmented landscape, the effect of individual traits responsible for species dependence on historical versus current landscape configuration remains unclear. We identified to what extent present (2000s) and historical (1843, 1954 and 1980s) landscape configuration affected species composition of dry calcareous grasslands. We used traits related to dispersal, survival, growth and habitat preference to explain dependence of 60 grassland species on present and historical habitat configuration. The composition of dry calcareous grasslands was affected by both area and connectivity in all time periods. Detailed analyses of species occurrences revealed that 69 % of species under study depended positively on habitat configuration, but almost half of them (44 %) depended only on habitat configuration in the past suggesting that many grassland species are not in equilibrium with the current landscape. Endozoochorous fast-growing species with higher seed bank longevity and requirements to nutrients were positively associated with habitat area and connectivity in the last 30 years. In contrast, epizoochorous slow-growing species with lower seed bank longevity and requirements to nutrients but higher requirements to light were positively associated with habitat area and connectivity up to 1954. These results indicate that the predictions of which species will profit from future changes will need to carefully consider not only change in the landscape structure, but also processes driving change in land-use and habitat quality.

Keywords: Bromion erecti, extinction debt, dispersal limitation, land-use, metapopulation dynamics

Introduction

Extensive changes in land use during the last few centuries have resulted in increased levels of habitat fragmentation for many plant species (Cousins 2001; Jacquemyn et al. 2003; Hérault and Honnay 2005; Piessens et al. 2005; Chýlová and Münzbergová 2008; Johansson et al. 2008). These changes have provoked discussion about the effects of fragmentation on species dynamics and distribution (Eriksson et al. 1995; Bastin and Thomas 1999; Dupré and Ehrlén 2002; Mildén et al. 2006; Cousins 2009; Knappová et al. 2012).

Dynamics of plant species in landscape are result of extinction and colonization rates (e.g., Harrison 1991; Eriksson 1996; Husband and Barrett 1996; Ehrlén and Eriksson 2000; Münzbergová et al. 2005). Habitat configuration and its changes can affect both extinction of local populations and colonization of available suitable habitats. Larger habitats can host larger populations which are more resistant to the negative effects of demographic and environmental stochasticity (e.g., Fischer and Stöcklin 1997; Matthies et al. 2004; Münzbergová 2006; Hemrová at al. 2012). Habitat size is also important for colonization rates as larger habitats are better accessible to species. In addition, colonization rates are also strongly affected by habitat isolation. Current habitat area and connectivity have been repeatedly shown to affect species occurrence (e.g., Eriksson et al. 1995; Bruun 2000; Dupré and Ehrlén 2002; Jacquemyn et al. 2003, Piessens et al. 2005; Lindborg et al. 2012). In contrast, there is also a lot of species for which the current habitat configuration has not been proven to be significant. Some of these species can, however, reflect historical habitat configuration due to dispersal limitation (Herben et al. 2006; Münzbergová et al. 2013) and extinction debt (Eriksson 1996). Such disequilibrium dynamics are very likely in many species of European agriculture landscape as a result of rapid landscape changes and slow species response to these changes.

A useful approach for understanding species dynamics in a changing landscape is to analyze the relationships between species occurrence in habitats with different configurations and species life-history traits related to dispersal and survival (e.g., Verheyen et al. 2004; Kolb and Diekmann 2005; Adriaens et al. 2006; Endels et al. 2007; Tremlová and Münzbergová 2007; Schleicher et al. 2011; Koyanagi et al. 2012). Earlier studies on the relationships between landscape configuration and species traits either have not used historical habitat configuration (e.g., Bruun 2000; Dupré and Ehrlén 2002; Kolb and Diekmann 2005; Tremlová and Münzbergová 2007; Schleicher et al. 2011; Lindborg et al. 2012) or have used the classification of species into emergent species groups according to their traits (e.g., Hérault and Honnay 2005; Adriaens et al. 2006; Endels et al. 2007; Koyanagi et al. 2012). Omitting historical habitat configuration can result in misleading conclusion that landscape configuration is not important to current species dynamics, whereas using emergent species groups instead of individual traits cannot answer the question how individual traits contribute to species dynamics in the landscape. Although several earlier studies have suggested that persistence traits relate to dependence of species on historical configuration (e.g., Hérault and Honnay 2005; Adriaens et al. 2006; Lindborg 2007), only a few similar findings have been made for species dispersal (but see Koyanagi et al. 2012; Purschke et al. 2012). Moreover, many previous studies have used a single dispersal mode for each individual species (e.g., Jacquemyn et al. 2003; Verheyen et al. 2004; Hérault and Honnay 2005; Adriaens et al. 2006; Endels et al. 2007; Lindborg 2007), although species can disperse by multiple vectors (Tackenberg et al. 2006; Bruun and Poschlod 2006; Adriaens et al. 2007). Therefore the effects of individual life-history traits, especially the dispersal traits, on species dynamics in the changing landscape remain unclear.

The aim of present study is to reveal the importance of present and historical habitat configuration for both species composition and occurrence of individual species and to

identify the relationships between life-history traits and species dependence on habitat configuration. Specifically, we asked the following questions: (i) Is the species composition in dry grasslands affected by current and/or historical habitat configuration? (ii) Which life-history traits are responsible for species dependence on habitat configuration at present and in the past? (3) Do the effects of traits responsible for species dependence on habitat configuration differ among time periods?

To answer these questions, we recorded occurrence of 99 dry grassland plant species in 371 dry calcareous grasslands. We estimated current habitat configuration (area and connectivity) of the grasslands at present and in the past (1843, 1954 and 1980s) and used it to explain the composition of the grasslands and the distribution of individual species. For a set of dry grassland species we also assembled data on life-history traits related to dispersal, growth, survival and habitat preference. The traits were used to explain the species dependence on habitat configuration in different time periods.

Methods

Study area and species

The study area (8 by 8 km) is situated in northern Bohemia in the Czech Republic (NW corner: 50°33'19"N, 14°15'6"E, SE corner: 50°29'45"N, 14°22'31"E; for details, see Knappová et al. 2012). Calcareous dry grasslands (alliance *Bromion erecti*, Ellenberg 1988) are typical of the landscape. These grasslands form distinct patches surrounded by shrubs and large agricultural fields. The dry grasslands were formerly managed, now they are abandoned and experience slow succession of shrubs and trees. In contrast, some dry grassland species have colonized fields abandoned in the last 20 years (Knappová et al. 2012).

Data collection

In 2009 we localized 371 grasslands in the area and recorded presence of 99 species on these grasslands (Table 1). For each grassland in the current landscape, we calculated its area and connectivity to surrounding grasslands at present and in the past. To calculate the area and connectivity of the grasslands at present, we used the information on current structure and distribution of the grasslands in the study area. The area and connectivity of the grasslands in the past were calculated based on the structure and distribution of land use plots in different time layers. Specifically, we used cadastral maps from 1843 (1:2 880) and special maps from 1954 and 1980s (1980-1986, 1:5 000) which combine topographical and cadastral information. All maps were provided by the Czech Office for Surveying, Mapping and Cadastre. The maps from 1954 were rectified for a previous study (Chýlová and Münzbergová 2008) and used for rectification of the maps from 1843 and 1980s in ArcGIS 9.3 (Environmental Systems Research Institute Inc. 2008). Based on the rectified maps, we created digital maps of pastures, meadows and uncultivated plots for each past time layer in the study area. We focused on the plots of pastures, meadows and uncultivated plots as the most common sources of dry grassland species. For each current grassland, we calculated its area at present (2000s) and the overall area of potential grassland habitats (pastures, meadows and uncultivated plots) in 1980s, 1954 and 1843 within the area of the given current grassland. The connectivity of the grasslands was expressed as the area of the grasslands (in 2000s) or potential grassland habitats (pastures, meadows and uncultivated plots in 1980s, 1954 and 1843) within the buffer of 500 m from the boundary of the given current grassland (similarly in Endels et al. 2007). The area in the past was expressed as the proportion of the current area of the grasslands covered by

the past potential grasslands. The area of the current grasslands was expressed as log transformed area in m^2. The connectivity at present and in the past was expressed as proportion of the buffer zone covered by the grassland. Seventy three grasslands were excluded from the further analyses, because their buffer exceeded boundary of the study area.

Table 1. – List of species under study, their abbreviations and incidence in 298 dry grasslands and effects (β) of area and connectivity of the grasslands in 1843, 1954, 1980s and 2000s on the presence of species under study with incidence ≥ 10.

Name	*Abbr.*	*Inc.*	*β* area				*β* connectivity			
			1843	*1954*	*1980s*	*2000s*	*1843*	*1954*	*1980s*	*2000s*
Agrimonia eupatoria	*Agr eup*	*274*	*-0.199*	*0.439*	*1.255**	*0.467*	*2.907*	*5.788*	*4.109*	*4.924*
Anemone sylvestris	*Ane syl*	*46*	*0.422*	*0.859*	*0.928**	*0.199*	*-2.874*	*1.336*	*-0.129*	*-3.077*
Anthericum ramosum	*Ant ram*	*21*	*1.323**	*0.686*	*-0.281*	*0.126*	*5.685*	*2.606*	*0.285*	*-2.695*
Anthyllis vulneraria	*Ant vul*	*69*	*1.742***	*2.057***	*0.754**	*0.409**	*3.048*	*8.442***	*-0.785*	*-6.543***
Artemisia campestris	*Art cam*	*5*								
Asperula cynanchica	*Asp cyn*	*47*	*1.291***	*1.159**	*0.925**	*0.102*	*2.823*	*5.412*	*1.746*	*-1.916*
Asperula tinctoria	*Asp tin*	*23*	*0.697*	*0.496*	*0.204*	*0.349*	*6.257*	*4.858*	*-1.407*	*-6.335*
Aster amellus	*Ast ame*	*27*	*0.301*	*1.607**	*1.1**	*0.616**	*-1.282*	*11.043***	*0.804*	*-2.595*
Aster linosyris	*Ast lin*	*15*	*0.774*	*0.763*	*-0.098*	*0.344*	*-1.538*	*2.214*	*2.087*	*-1.282*
Astragalus cicer	*Ast cic*	*114*	*-0.423*	*-1.22**	*0.073*	*0.685***	*4.17**	*-4.777*	*-0.209*	*3.552**
Astragalus glycyphyllos	*Ast gly*	*182*	*-1.18***	*-1.298***	*0.834***	*1.004***	*6.632***	*1.626*	*5.392***	*3.566**
Brachypodium pinnatum	*Bra pin*	*251*	*-0.164*	*-0.696*	*0.918**	*0.146*	*4.678*	*-3.043*	*2.96*	*2.026*
Briza media	*Bri med*	*132*	*0.02*	*0.362*	*0.651**	*0.498***	*3.438*	*9.678***	*2.294**	*-0.569*
Bromus erectus	*Bro ere*	*126*	*1.472***	*2.168***	*0.854***	*0.342**	*4.545**	*9.6***	*1.973*	*-1.128*
Bupleurum falcatum	*Bup fal*	*200*	*0.692*	*0.975*	*0.461*	*0.38**	*2.16*	*11.092***	*2.024*	*-3.186*
Campanula glomerata	*Cam glo*	*2*								
Campanula rotundifolia	*Cam rot*	*9*								
Carex flacca	*Car fla*	*122*	*0.064*	*0.436*	*0.534*	*0.407**	*1.83*	*2.949*	*1.816*	*1.378*
Carex humilis	*Car hum*	*49*	*0.963**	*-0.246*	*0.6*	*0.269*	*8.595***	*7.563**	*5.014***	*-3.644*
Carex tomentosa	*Car tom*	*73*	*0.753*	*0.284*	*0.618*	*0.775***	*4.379**	*-2.191*	*-0.127*	*4.285**
Carlina vulgaris	*Car vul*	*84*	*0.842**	*0.507*	*0.039*	*0.76***	*2.174*	*2.114*	*-1.243*	*0.002*
Centaurea jacea	*Cen jac*	*210*	*1.263**	*0.29*	*0.423*	*1.055***	*8.703***	*2.076*	*2.172*	*3.299*
Centaurea rhenana	*Cen rhe*	*7*								
Centaurea scabiosa	*Cen sca*	*193*	*0.159*	*0.562*	*0.908***	*0.619***	*1.311*	*8.939***	*3.713***	*-4.684**
Cirsium acaule	*Cir aca*	*87*	*1.083**	*1.352***	*0.58*	*0.423**	*1.555*	*6.274**	*0.583*	*-0.346*
Cirsium eriophorum	*Cir eri*	*31*	*-0.49*	*-2.031**	*0.053*	*1.214***	*11.489***	*-9.187**	*5.316***	*15.558***
Cirsium pannonicum	*Cir pan*	*10*	*-1.593*	*-1.278*	*0.487*	*0.572*	*8.382*	*4.748*	*11.193***	*10.634***
Coronilla vaginalis	*Cor vag*	*3*								
Coronilla varia	*Cor var*	*264*	*-0.826*	*-0.251*	*0.888**	*0.791***	*-0.84*	*7.566*	*2.916*	*-5.445**
Dianthus carthusianorum	*Dia car*	*45*	*0.233*	*0.104*	*0.643*	*0.531**	*3.854*	*10.199***	*1.786*	*-5.845**
Eryngium campestre	*Ery cam*	*137*	*1.586***	*1.339***	*0.677**	*0.209*	*0.926*	*4.213*	*-0.04*	*-4.645**
Euphrasia rostkoviana	*Eup ros*	*14*	*-0.173*	*-0.351*	*0.288*	*1.091***	*3.246*	*1.969*	*0.56*	*1.974*
Falcaria vulgaris	*Fal vul*	*140*	*0.05*	*0.317*	*-0.137*	*0.539***	*0.593*	*6.017**	*-0.465*	*-2.829*
Festuca rupicola	*Fes rup*	*226*	*0.455*	*0.882*	*1.091***	*0.671***	*3.396*	*9.149***	*2.757**	*-2.548*
Filipendula vulgaris	*Fil vul*	*11*	*1.513*	*1.279*	*-0.144*	*-0.399*	*10.173**	*2.372*	*-6.005*	*7.353**
Fragaria viridis	*Fra vir*	*250*	*0.048*	*-0.438*	*0.426*	*0.798***	*3.26*	*6.343*	*1.679*	*-1.227*
Galium verum	*Gal ver*	*220*	*-0.474*	*-0.583*	*-0.398*	*0.525***	*0.034*	*5.097*	*-1.377*	*0.419*
Genista tinctoria	*Gen tin*	*29*	*-0.317*	*-1.276*	*0.049*	*0.228*	*6.277**	*7.666*	*4.929***	*-5.14*
Gentiana cruciata	*Gen cru*	*61*	*-0.259*	*-0.048*	*0.164*	*0.648***	*-1.211*	*3.283*	*-0.921*	*-2.416*
Geranium sanguineum	*Ger san*	*27*	*0.274*	*-0.751*	*0.353*	*0.243*	*4.54*	*3.187*	*3.841**	*-0.132*
Globularia elongata	*Glo pun*	*15*	*0.729*	*0.746*	*0.674*	*0.718*	*6.856*	*7.702*	*5.374**	*1.047*
Gymnadenia conopsea	*Gym con*	*3*								
Helianthemum nummularium subsp. grandiflorum	*Hel gra*	*60*	*-0.585*	*-0.284*	*0.987***	*0.253*	*4.939**	*8.54***	*5.785***	*-5.136**
Hieracium pilosella	*Hie pil*	*76*	*0.582*	*0.33*	*1.018***	*0.617***	*3.044*	*4.884*	*3.067**	*-3.682*
Hypericum perforatum	*Hyp per*	*218*	*-0.536*	*0.246*	*-0.55*	*0.233*	*-0.846*	*4.772*	*-1.581*	*-2.233*
Inula hirta	*Inu hir*	*13*	*0.58*	*0.217*	*-0.833*	*0.238*	*9.345**	*2.004*	*-1.531*	*-5.974*
Inula salicina	*Inu sal*	*207*	*0.612*	*0.126*	*0.27*	*1.062***	*3.225*	*-2.555*	*0.092*	*4.55**

* $P < 0.05$; ** $P < 0.05/8$ (Bonferroni correction)

Table 1 continued

Name	Abbr.	Inc.	β area				β connectivity			
			1843	1954	1980s	2000s	1843	1954	1980s	2000s
Knautia arvensis	Kna arv	258	1.045	2.428*	0.535	-0.086	3.482	1.425	0.331	-2.305
Koeleria pyramidata	Koe pyr	61	0.477	0.507	0.351	0.207	0.762	1.824	-0.539	0.098
Laserpitium latifolium	Las lat	2								
Lathyrus pratensis	Lat pra	77	-0.904	-0.963	-0.137	0.437*	5.152*	-2.116	1.226	8.143**
Leontodon hispidus	Leo his	150	0.439	0.276	0.682*	0.989**	2.114	2.508	1.713	-0.676
Leucanthemum vulgare	Leu vul	81	-0.446	-0.078	0.62*	0.779**	2.761	4.224	4.148**	4.103*
Linum catharticum	Lin cat	206	0.392	0.641	0.612*	0.963**	3.552	6.073*	1.705	-0.252
Linum flavum	Lin fla	10	-0.936	-0.07	0.799	0.618	-3.087	4.549	9.622**	0.244
Linum tenuifolium	Lin ten	15	1.183	2.177**	0.739	0.563	5.241	2.611	0.821	3.522
Listera ovata	Lis ova	10	1.369	1.629	0.968	0.47	-0.33	-6.936	-2.855	5.752
Lotus corniculatus	Lot cor	234	-0.038	-0.438	1.136**	1.025**	2.641	2.759	4.104**	2.48
Medicago falcata	Med fal	111	1.367**	1.22**	0.684*	0.058	1.64	2.873	-0.378	-0.96
Melampyrum arvense	Mel arv	25	0.048	0.715	0.787	0.809**	-4.607	-1.585	-1.225	0.456
Melampyrum cristatum	Mel cri	2								
Melampyrum nemorosum	Mel nem	16	-0.169	-2.69	0	0.274	10.162*	-2.613	4.337	8.535**
Onobrychis viciifolia	Ono vic	10	1.493	1.367	1.931*	0.856	-2.528	-1.981	-3.142	2.245
Ononis spinosa	Ono spi	64	1.383**	1.048*	0.866*	0.581**	7.335**	1.013	1.209	5.005*
Origanum vulgare	Ori vul	151	-0.62	-1.016*	0.599*	0.622**	1.913	3.501	4.253**	-3.624*
Peucedanum cervaria	Peu cer	44	-0.02	-0.166	0.505	0.414	5.434*	10.534**	4.441**	-2.348
Peucedanum oreoselinum	Peu ore	11	0.095	-0.575	-0.115	0.009	11.03*	18.413**	0.604	-12.901
Pimpinella saxifraga	Pim sax	143	0.662	-0.068	0.889**	0.333*	1.11	4.751	0.075	-5.094**
Plantago lanceolata	Pla lan	193	-0.753	-0.224	0.623*	0.311	1.115	5.185	5.399**	0.516
Plantago media	Pla med	196	-0.056	-0.069	1.28**	0.938**	2.685	7.5**	2.665*	-1.591
Platanthera bifolia	Pla bif	19	-0.339	-1.723	0.897	0.394	-3.051	-2.55	1.369	-5.314
Potentilla arenaria	Pot are	35	1.421**	0.86	0.902*	0.426	3.765	2.193	1.978	-3.841
Potentilla heptaphylla	Pot hep	123	-0.351	-0.573	0.402	0.085	-0.362	3.542	1.082	-2.519
Primula veris	Pri ver	94	-0.67	-0.489	0.255	0.448*	0.317	4.111	1.738	1.106
Prunella grandiflora	Pru gra	90	0.42	-0.093	0.373	0.269	2.878	6.077*	2.042	-3.984*
Prunella vulgaris	Pru vul	101	-1.232**	-2.079**	0.052	0.605**	7.661**	2.662	5.937**	5.593**
Pulsatilla pratensis subsp. bohemica	Pul pra	3								
Salvia nemorosa	Sal nem	43	-0.253	1.391*	0.502	0.542*	1.303	9.714**	-0.895	-2.551
Salvia pratensis	Sal pra	195	1.447**	1.087*	0.986**	0.345*	5.772*	6.356*	3.579**	-2.651
Salvia verticillata	Sal ver	154	0.713	1.522**	0.708*	0.279	-0.145	1.386	-0.337	0.222
Sanguisorba minor	San min	207	1.436**	0.838	0.853**	0.267	3.814	7.223*	1.208	-3.589*
Scabiosa canescens	Sca can	1								
Scabiosa ochroleuca	Sca och	156	0.43	1.095*	1.024**	0.382*	1.508	8.476**	1.731	-6.361**
Scorzonera hispanica	Sco his	4								
Seseli hippomarathrum	Ses hip	7								
Sesleria albicans	Ses alb	20	0.77	-0.532	1.424*	0.525	3.103	4.308	2.917	0.524
Silene vulgaris	Sil vul	118	-0.272	-0.467	0.377	0.522**	0.139	3.338	0.595	-3.406
Solidago virgaurea	Sol vir	28	-0.374	-0.806	-0.199	0.394	0.466	5.909	1.875	-0.309
Stachys recta	Sta rec	108	0.63	1.184*	0.376	0.351*	-0.251	5.482*	0.503	-2.763
Tanacetum corymbosum	Tan cor	46	0.679	-0.051	-0.196	0.479*	3.185	-0.882	1.635	2.748
Teucrium chamaedrys	Teu cha	64	0.766	0.361	0.219	0.001	3.304	4.461	1.546	-4.88*
Thesium linophyllon	The lin	4								
Thymus praecox	Thy pra	79	1.084*	1.92**	1.147**	0.191	2.57	7.474**	1.908	-5.378*
Trifolium medium	Tri med	191	-0.854*	-0.539	1.071**	0.581**	2.409	0.288	5.562**	3.991*
Trifolium montanum	Tri mon	46	-0.209	0.783	0.261	0.091	0.482	9.245**	2.048	-9.647**
Veronica austriaca subsp. teucrium	Ver teu	35	-0.391	0.003	1.2*	0.208	-2.447	4.374	1.257	-6.619*
Vicia cracca	Vic cra	159	-0.494	-0.211	0.651*	0.559**	0.627	2.352	3.503**	2.246
Vicia sepium	Vic sep	75	-0.98*	-0.317	0.322	0.606**	5.063*	2.09	2.898*	5.296**
Viola hirta	Vio hir	63	0.368	-0.506	0.233	0.222	0.995	-3.725	-1.174	-1.85

* $P < 0.05$; ** $P < 0.05/8$ (Bonferroni correction)

The nomenclature follows Tutin et al. (1964–1983).

We assembled data on species traits related to species dispersal ability, survival, growth and habitat preference (Table 2). These included 1) traits related to dispersal – terminal velocity, rate of epizoochory (i.e. ability to attach to sheep fur) and rate of endozoochory (i.e. ability to survive endozoochory), 2) traits related to both dispersal and survival/growth – seed mass and plant height, 3) traits related to survival/growth – seed bank longevity and specific leaf area and 4) traits describing species habitat requirements – Ellenberg indicator values for light and nutrients (Ellenberg 1988). The data on terminal velocity, rate of epizoochory, rate of endozoochory, seed mass and plant height for 85 species were assembled in previous study by Tremlová and Münzbergová (2007) and in Hemrová et al. (**Chapter 4**). The data on seed bank longevity and specific leaf area were obtained from LEDA database (Kleyer et al. 2008). We calculated mean value of records of specific leaf area for each species and seed bank longevity index according to Bekker et al. (1998). Values of seed mass and plant height were logarithmically transformed before analyses.

Table 2. – List of traits under study, trait units and mean and range of trait values in 60 species used in trait analyses.

Trait	Units	Mean	Range
Light	category 1-9	7.17	5.00-9.00
Nutrients	category 1-9	2.97	1.00-6.00
Plant height	log(m)	-0.41	-1.12-0.02
Rate of endozoochory	%	68.51	0.00-100.00
Rate of epizoochory	%	15.75	0.00-80.00
Seed bank longevity	index	0.13	0.00-0.75
Seed mass	log(mg)	0.02	-3.31-1.43
Specific leaf area	mm^2/mg	20.89	9.31-37.86
Terminal velocity	m/s	2.15	0.26-3.37

Data analysis

First, we estimated the impact of current and past habitat configuration on species composition of the grasslands using canonical correspondence analyses (CCA, ter Braak 1986). Presences/absences of the species under study were used as dependent variables, the area and connectivity of the given grassland in 2000s, 1980s, 1954 and 1843 were used as independent variables. The area and connectivity as well as each time layer were tested separately. We also tested the effect of area and the effect of connectivity in 1980s, 1954 and 1843 using the area/connectivity in all subsequent time layers as covariates.

To test the effect of habitat configuration on occurrence of a single species, we used generalized linear models (GLMs) assuming binomial distribution of dependent variables. Presence/absence of the target species at the locality were used as dependent variables. The area and connectivity of the grasslands in 2000s, 1980s, 1954 and 1843 were used as independent variables and tested separately. The species with incidence < 10 were excluded from these analyses (Table 1). Due to the high number of independent analyses we used Bonferroni correction to modify the significance value. Then we tested the effect of traits on species dependence on habitat configuration; the dependence was expressed by regression coefficients (β area and β connectivity in 2000s, 1980s, 1954 and 1843) derived from the previous GLMs analyses (similarly Dupré and Ehrlén 2002). Relationships between species traits and β area and β connectivity in individual time layers as well as correlations among traits were analyzed by Spearman's rank correlations; for correlations among species traits see Appendix 2 in **Chapter 4**. From trait analyses we excluded

species with missing trait values and finally used 60 species in these analyses. All multivariate analyses were performed using CANOCO (ter Braak and Šmilauer 1998), the univariate analyses were performed using R 2.14.1 (R Development Core Team 2011).

Table 3. – Effects of area and connectivity of dry grasslands in 1843, 1954, 1980s and 2000s on species composition. Significant effects ($p < 0.05$) are in **bold**.

Variable	No covariate		With covariate		
	P	R^2	Covariate	P	R^2
Area					
1843	**0.002**	**0.012**	Area 2000s, 1980s, 1954	**0.002**	**0.007**
1954	**0.002**	**0.011**	Area 2000s, 1980s	**0.002**	**0.011**
1980s	**0.010**	**0.006**	Area 2000s	**0.010**	**0.006**
2000s	**0.002**	**0.009**			
Connectivity					
1843	**0.002**	**0.007**	Connectivity 2000s, 1980s, 1954	**0.004**	**0.006**
1954	**0.002**	**0.007**	Connectivity 2000s, 1980s	**0.018**	**0.005**
1980s	**0.002**	**0.010**	Connectivity 2000s	**0.002**	**0.011**
2000s	**0.002**	**0.016**			

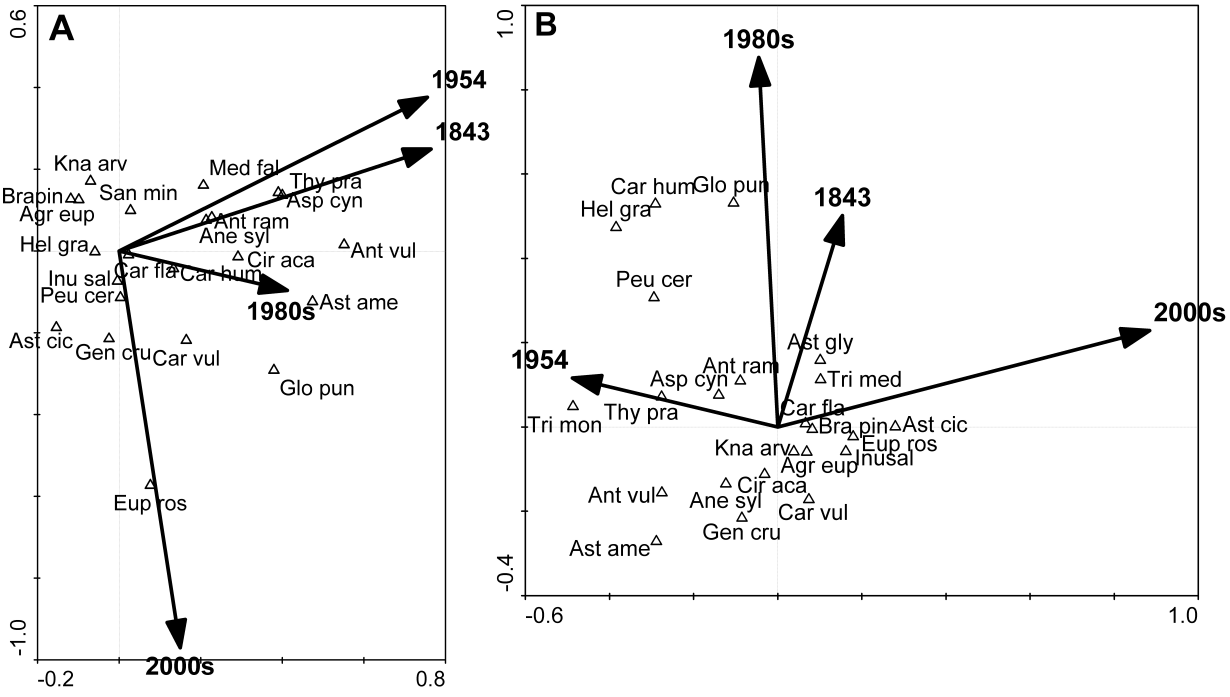


Fig. 1. – Effects of habitat (A) area and (B) connectivity in 1843, 1954, 1980s and 2000s on the occurrence of the studied dry grassland species in habitats estimated using CCA analyses (see also **Table 3**). Habitat configuration in 1954, 1980s and 2000s were used as covariates in both cases. For species abbreviations see **Table 1**.

Results

The species composition of dry grasslands significantly depended ($P < 0.05$) on both their area and their connectivity in 1843, 1954, 1980s and 2000s (Table 3, Fig. 1). The relationships remained significant even though the subsequent time layers were used as

covariates (Table 3). The species restricted to larger habitats in the past were e.g., *Asperula cynanchica*, *Aster amellus*, *Carex humilis*, *Medicago falcata*, *Thymus praecox*, whereas the species restricted to current larger habitats were e.g., *Astragalus cicer*, *Euphrasia rostkoviana*, *Gentiana cruciata* (Fig. 1A). The species restricted to habitats with higher historical connectivity were e.g., *Carex humilis*, *Helianthemum grandiflorum*, *Trifolium montanum*, *Peucedanum cervaria*, whereas species restricted to habitat with higher connectivity at present were e.g., *Astragalus cicer*, *Euphrasia rostkoviana*, *Inula salicina* (Fig. 1B).

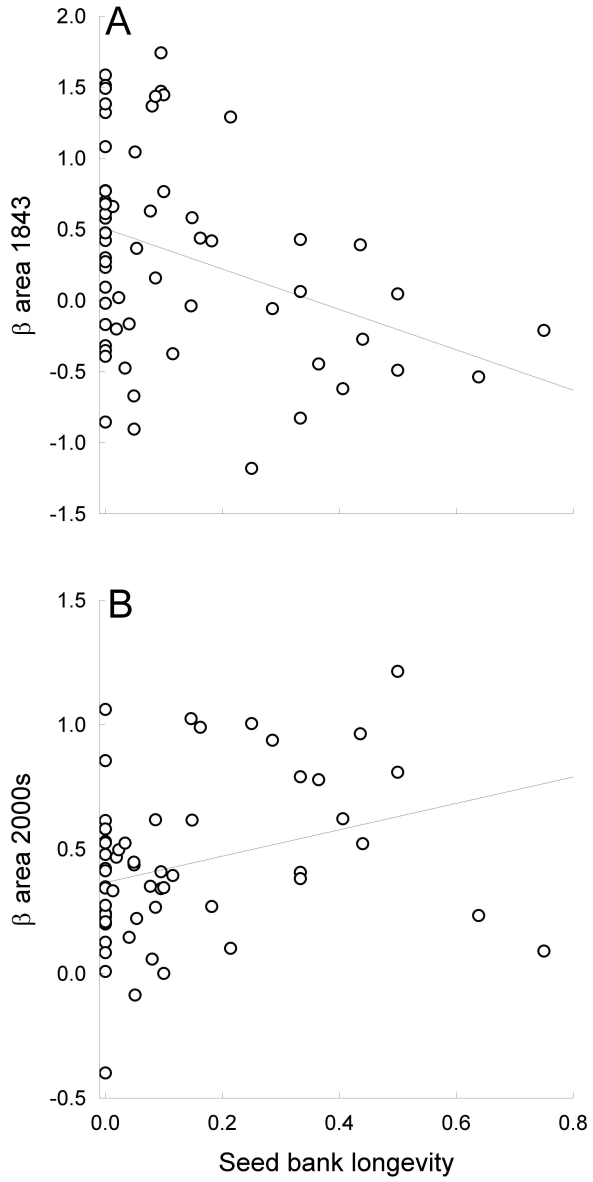


Fig. 2. – Relationships between seed bank longevity of dry grassland species and their response to the area of dry grassland habitats (A) in 1843 and (B) in 2000s. Species response to habitat area is derived from logistic regression analyses as regression coefficients (β).

Fifty nine out of 86 species significantly positively depended on any area or connectivity ($P < 0.05/8$ after Bonferroni correction). Twenty-eight percent of the tested species depended on habitat area only, 16 % on habitat isolation only, 20 % of the species depended on habitat configuration at present only, 30% in past only. Specifically, we found 30 species to significantly positively depend on the area of grasslands in 2000s, 14 in

1980s, 8 in 1954 and 9 in 1843 (Table 1). In the case of connectivity we found 6 species to significantly positively depend on the connectivity of grasslands in 2000s, 17 in 1980s, 16 in 1954 and 6 in 1843 (Table 1).

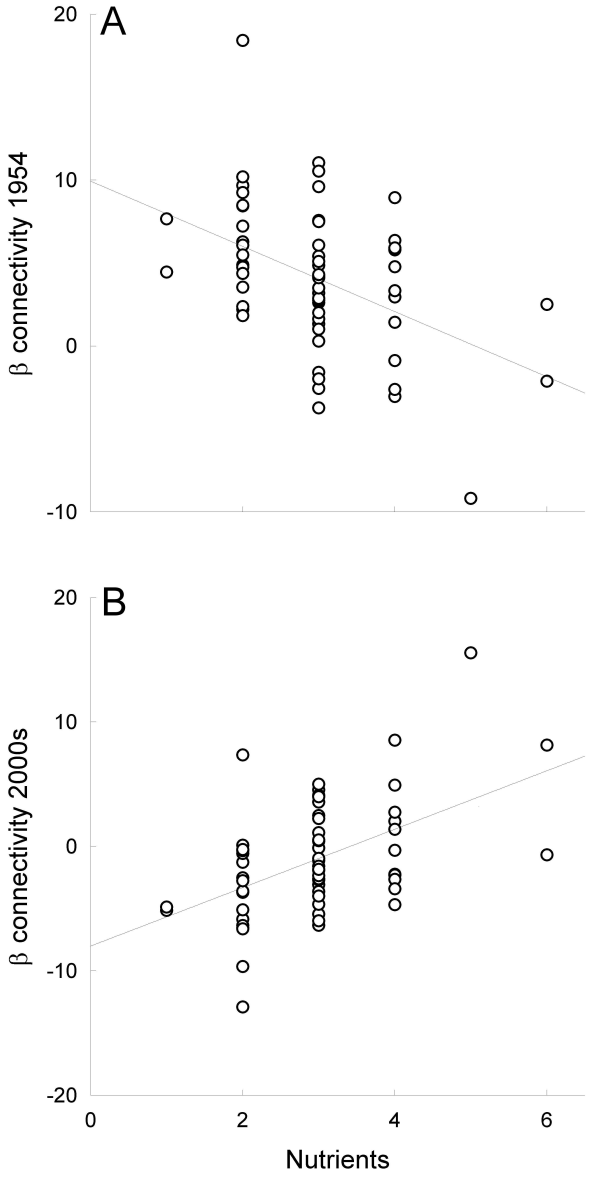


Fig. 3. – Relationships between requirements of dry grassland species for nutrients and their response to the connectivity of dry grassland habitats (A) in 1954 and (B) in 2000s. Species response to habitat connectivity is derived from logistic regression analyses as regression coefficients (β).

We found β area to be significantly ($P < 0.05$) positively correlated to light and negatively to seed bank longevity in 1843 (Fig. 2A), positively to light and the rate of epizoochory in 1954, non-correlated to any trait in 1980s and positively correlated to nutrients, the rate of endozoochory, seed bank longevity (Fig. 2B) and specific leaf area in 2000s (Table 4). In case of β connectivity we found positive correlations to plant height and seed mass in 1843, negative to nutrients in 1954 (Fig. 3A), positive to the rate of endozoochory in 1980s and positive to nutrients in 2000s (Table 4, Fig. 3B).

Table 4. – Relationships (r) between plant response to habitat configuration and their life-history traits as tested by Spearman's rank correlation analyses (N = 60). Species response to habitat configuration is derived from logistic regression analyses as regression coefficients (β) for area and connectivity.

Trait	β area				β connectivity			
	1843	1954	1980s	2000s	1843	1954	1980s	2000s
Light	**0.33***	**0.49*****	0.22	0.16	-0.14	0.22	-0.07	-0.12
Nutrients	-0.24	-0.23	-0.03	**0.26***	0.07	**-0.40****	0.16	**0.46*****
Plant height	-0.04	0.03	-0.05	-0.01	**0.30***	0.09	0.19	0.13
Rate of endozoochory	-0.08	-0.02	0.23	**0.26***	0.14	0.03	**0.37****	0.15
Rate of epizoochory	0.23	**0.32***	0.12	0.06	-0.11	-0.19	-0.14	0.18
Seed bank longevity	**-0.30***	-0.04	0.10	**0.28***	-0.17	0.14	0.22	-0.05
Seed mass	0.07	0.03	0.16	0.07	**0.27***	-0.02	0.22	0.13
Specific leaf area	-0.24	-0.18	0.04	**0.27***	0.08	-0.22	0.07	0.21
Terminal velocity	-0.12	-0.15	0.11	-0.11	0.09	-0.08	0.15	0.17

Discussion

In this study, we confirmed the importance of landscape configuration for the occurrence of most species of dry calcareous grasslands in central European landscape. We found that different traits are responsible for species dependence on habitat configuration in different time periods. Current composition of the grasslands is affected by habitat area and connectivity both at present (2000s) and in the past (1980s, 1954 and1843). Habitat area and isolation have been hypothesized to generally affect species occurrence (MacArthur and Wilson 1967). Despite that, many studies have found no effect of habitat configuration on species richness (Eriksson et al. 1995; Kiviniemi and Eriksson 1999; Pärtel and Zobel 1999), population sizes (Eriksson and Ehrlén 2001; Bruun 2005), or occurrence of individual species (Eriksson et al. 1995; Dupré and Ehrlén 2002; Piessens et al. 2005). The occurrence of 69 % of the species under study depended positively on habitat configuration, almost half of them (44 %), however, depended only on configuration in the past. Thirty-eight percent of the species depending on current habitat configuration (35% on area and 7% on connectivity) is in accord with the results of previous studies that have found between 4–57% of species depending on habitat area and between 5–64% depending on habitat connectivity (Eriksson et al. 1995; Bruun 2000; Dupré and Ehrlén 2002; Verheyen et al. 2004; Piessens et al. 2005; De Sanctis et al. 2010). Lindborg et al. (2012) have suggested that the importance of habitat configuration to forest and grassland species may differ finding 50% and 50% of forest species, whereas only 18% and 23% of grassland species depended on area and connectivity in their study, respectively.

The absence of the effect of current habitat configuration on species occurrence can have two explanations. First, the species is not dispersal limited in context of the studied landscape and all habitats have similar chance to be colonized irrespective of their area or isolation. Second, the species persists on changed habitats due to extinction debt (Eriksson 1996) and therefore reflects landscape configuration in the past. Species dependence on the past landscape configuration is very likely in European landscape due to extensive land-use changes and habitat fragmentation on the one hand (e.g., Bouma et al. 1998; Cousins 2001; Chýlová and Münzbergová 2008), and species slow response to these changes on the other hand (e.g., Jacquemyn et al. 2003; Ehrlén et al. 2006; Herben et al. 2006).

The species in the present study more often depended on habitat area than on habitat isolation. It could relate to the calculation of isolation in terms of the area of surrounding grassland habitats and to the fact that source populations of individual species were not considered. We, however, suppose that method of calculation of habitat connectivity did not considerably influence the results of the present study, because higher importance of

area than connectivity has also been found in other studies using other measures of habitat isolation (e.g., Verheyen et al. 2004; De Sanctis et al. 2010). Higher importance of habitat area is due to its effect not only on dispersal but also on species survival, because persistent traits such as seed bank longevity and specific leaf area were important to the dependence of the species under study on habitat area.

The species affected only by current habitat configuration in the present study such as *Astragalus cicer*, *Carlina vulgaris*, *Coronilla varia*, *Euphrasia rostkoviana*, *Fragaria viridis*, *Gentiana cruciata* and *Inula salicina* are often found on fields abandoned in the area in the last 20 years (Knappová et al. 2012). In contrast, the species affected only by historical habitat configuration such as *Anthyllis vulneraria*, *Asperula cynanchica*, *Aster amellus*, *Carex humilis*, *Cirsium acaule*, *Helianthemum grandiflorum*, *Medicago falcata*, *Peucedanum cervaria*, *Sanguisorba minor*, *Thymus praecox* and *Trifolium montanum* are rather found on older dry grasslands (Knappová et al. 2012) and are positively associated with forests in 1980s or grasslands in 1950s (Chýlová and Münzbergová 2008). The one third of species that was affected neither by present nor by historical habitat configuration include both common species such as *Agrimonia eupatoria*, *Brachypodium pinnatum*, *Carex flacca*, *Knautia arvensis* and rare species such as *Anemone sylvestris*, *Anthericum ramosum*, *Filipendula vulgaris*, *Globularia elongata*, *Onobrychis vicifolia*.

The difference in species dependence on habitat configuration is often attributed to different sets of life-history traits (e.g., Dupré and Ehrlén 2002; Verheyen et al. 2004; Hérault and Honnay 2005; Tremlová and Münzbergová 2007; Lindborg et al. 2012). In the present study, different traits were responsible for the species dependence on habitat configuration in different time periods. We however found clear trend in traits responsible for the species dependence on habitat area before and after 1954. Habitats that were large in the past (1843 and 1954) currently host epizoochorous slow-growing species with lower seed bank longevity and lower requirements to nutrients but higher to light. In contrast, large habitats in the last 30 years (i.e. in 2000s and 1980s) host endozoochorous fast-growing species with higher seed bank longevity and requirements to nutrients.

The divergence in traits of the species occupying large habitats before and after 1954 can be caused by dramatic change in land-use in the landscape such as land expropriation (e.g., Bičík et al. 2001; Václavík and Rogan 2009) and intensification of agriculture (e.g., Bouma et al. 1998; Rabbinge and van Diepen 2000; Václavík and Rogan 2009) in the 1950's. Fertilization has been found to have a negative effect on the occurrence of some grassland species (e.g., Eriksson et al. 1995). Grasslands established on abandoned fields after the intensification can therefore possess different habitat quality for example in nutrient availability and be unsuitable for some grassland species. Different habitat quality can consequently lead to the occurrence of species with different set of traits ensuring success under high competition, such as fast growth, increased requirements to nutrients and decreased requirements to light.

We found a clear difference in strategies to survive in the landscape between the species depending on historical vs. recent habitat configuration. The species positively depending on historical habitat configuration persist in the dry grasslands due to their slow-growing strategy, whereas the species positively depending on habitat configuration in the last 30 years persist in the dry grasslands due to longevity of their seed bank. This finding is opposite to Purschke et al. (2012) who identify seed bank longevity to decrease and plant longevity to increase in their importance in species depending on current vs. historical habitat configuration. No linkages between dispersal traits in current communities and historical landscape configurations have been detected in earlier studies (e.g., Hérault and Honnay 2005; Adriaens et al. 2006; Lindborg 2007). In contrast to these studies and similarly to Purschke et al. (2012), we identified epizoochory as important to species occurrence on historical habitats. In contrast to Purschke et al. (2012) we, however, identified endozoochory as the type of long-distance dispersal important for species

occurrence under current landscape configuration. Higher importance of epizoochory up to 1954 versus higher importance of endozoochory after 1954 can relate to the exchange of extensive sheep grazing by grazing of wild animals and more intensive grazing of cattle, since Tackenberg et al. (2006) have shown importance of skin type on retention potential of seeds.

Conclusions

Habitat configuration is considered to be an important factor affecting species occurrence. In the present study, we found that the composition of dry calcareous grasslands is affected not only by current but also by historical habitat configuration even in the 19th century. Detailed analyses of species occurrences revealed that 69 % of the species under study depend positively on habitat configuration. At the same time we also found that almost half of these species (i.e. 30 % of all species) depend only on configuration in the past. It suggests that the distribution of many grassland species is not in equilibrium with the current landscape.

The species responding to current and past landscape configuration differed in their traits. The epizoochorous slow-growing species with lower seed bank longevity and requirements to nutrients but higher requirement to light were associated with larger habitats up to 1954, whereas the endozoochorous fast-growing species with higher seed bank longevity and requirements to nutrients were associated with larger habitats in the last 30 years. These results indicate that the relationship between species traits and landscape structure strongly depends on the quality of habitats in specific time periods as well as on other factors such as specific type of land use. Due to this, predictions of which species will profit from future changes in the landscape will need to carefully consider not only the overall change in the structure but also the details of the processes driving this change.

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Chapter 4

Age and number of suitable habitats and seed bank longevity as determinants of habitat occupancy

Lucie Hemrová, Dana Průchová and Zuzana Münzbergová

Abstract

A number of studies show the effect of life-history traits on habitat occupancy (ratio of occupied habitats) in cross-species analyses. Only few studies, however, focus in an analogous way on the effect of mean age and overall number of suitable habitats on habitat occupancy, although both habitat age and isolation were found to have impact on distribution of individual species. The relationship among the age of habitats suitable for a given species, the isolation of habitats suitable for a given species, species traits and habitat occupancy thus remains unclear. We identified suitable habitats for a set of dry grassland species in a current landscape. Based on the information from old maps (1843, 1954 and 1980s), we estimated age of each current dry grassland habitat. We used the mean age and the total number of suitable habitats of each species and traits related to dispersal, survival, growth and habitat preference to explain difference in habitat occupancy among 60 dry grassland species. We found seed bank longevity and the number of suitable habitats to have a positive effect on habitat occupancy, while the mean age of suitable habitats was found to have a negative effect on habitat occupancy. We also found negative relationship between the number and age of suitable habitats of the species. Species occupying younger habitats had higher specific leaf area and requirements to nutrients which suggests a difference in habitat quality between younger and older habitats. These results indicate that availability of suitable habitats is a major factor determining species habitat occupancy. The number of suitable habitats is, however, influenced by species traits such as species requirements to nutrients and specific leaf area; fast-growing competitive species have more suitable habitats as they include both older and younger habitats among those that are suitable for them. Although we identified seed bank longevity as the only important species trait explaining habitat occupancy, requirements to nutrients and specific leaf area affect habitat occupancy indirectly through their impact on the number of habitats suitable for given species.

Keywords: Bromion erecti, colonization, species growth rate, land-use, metapopulation dynamics, unoccupied habitats

Introduction

Determinants of species dynamics in fragmented landscape has been one of the most important ecological themes in the last decades. Many studies have shown that a number of habitats suitable for a plant species in a landscape stay unoccupied (e.g., Turnbull et al. 2000; Münzbergová 2004; Ehrlén et al. 2006). The differences in habitat occupancy (i.e. the ratio of occupied habitats) among species are hypothesized to be due to landscape properties on the one side (Levins 1969; Hanski and Gyllenberg 1993) and species colonization and survival ability on the other side (e.g., Eriksson and Jakobsson 1998; Piessens et al. 2005; Tremlová and Münzbergová 2007).

Landscape properties such as habitat age and isolation are very often used to assess their impact on species composition, richness and occurrence of individual species (e.g., Eriksson et al. 1995; Dupré and Ehrlén 2002; Lindborg and Eriksson 2004; Adriaens et al. 2006; Endels et al. 2007; Johansson et al. 2008). Only few studies, however, use the overall isolation of all habitats of a given species (e.g., Bastin and Thomas 1999; Adriaens et al. 2007) and no studies use the overall age of the habitats of given species in cross-species analyses of habitat occupancy. In contrast, species colonization and survival ability are very often used to explain habitat occupancy (e.g., Eriksson and Jakobsson 1998; Kiviniemi and Eriksson 1999; Ehrlén and Eriksson 2000; Jacquemyn et al. 2003; Maurer et al. 2003; Münzbergová 2004; Piessens et al. 2005; Adriaens et al. 2007). Colonization and survival ability are expressed by a wide range of life-history traits such as seed mass, seed production, dispersal mode, plant height and seed bank longevity (e.g., Murray et al. 2002; Van der Veken et al. 2007; Kleyer et al. 2008). Previous studies, however, differ in their conclusions about the effect of individual traits on habitat occupancy. Moreover, previous studies have used a single dispersal mode (except e.g., Kiviniemi and Eriksson 1999; Münzbergová 2004; Tremlová and Münzbergová 2007 and Adriaens et al. 2007), although species can disperse by multiple vectors (Bruun and Poschlod 2006; Tackenberg et al. 2006). Therefore the relationship among the age of habitats suitable for a given species, the isolation of habitats suitable for a given species, species traits and habitat occupancy remains unclear.

The aim of the present study is to reveal the impact of selected traits related to species dispersal and survival, the mean age of habitats suitable for a given species and the overall isolation of habitats suitable for a given species on habitat occupancy of the species. We asked the following questions: (i) What species life-history traits are responsible for differences in habitat occupancy among studied species? (ii) What is the effect of the overall age and number of suitable habitats (taken as the measure of habitat isolation) of each species on species habitat occupancy? (iii) Is there any correlation between species traits and number and overall age of habitats occupied by each species? We hypothesize that habitat occupancy increases with habitat number as it increases migration rate. We also hypothesize that some of the studied life-history traits have a positive effect on habitat occupancy.

To answer the questions, we estimated habitat suitability for 85 dry grassland species in a landscape. We calculated the mean age of suitable habitats of individual species. We used the number of suitable habitats of each species as the measure of habitat isolation assuming that connectivity among habitats increases with their number in the landscape (similarly Bastin and Thomas 1999). We also assembled data on species life-history traits related to dispersal, survival, growth and habitat preferences. Then we used habitat age, habitat number and species traits to explain differences in habitat occupancy among the studied species.

Methods

Study area and species

The study area (8 by 8 km) is situated in northern Bohemia in the Czech Republic (NW corner: 50°33'19"N, 14°15'6"E, SE corner: 50°29'45"N, 14°22'31"E; for details, see Knappová et al. 2012). Calcareous dry grasslands (alliance *Bromion erecti*, Ellenberg 1988) are typical of the landscape. These grasslands form distinct patches surrounded by shrubs and large agricultural fields. The dry grasslands were formerly managed, now they are abandoned and experience slow succession of shrubs and trees. In contrast, some dry grassland species have colonized fields abandoned in the last 20 years (Knappová et al. 2012). Based on studies performed within the same region (Münzbergová 2004; Tremlová and Münzbergová 2007; Chýlová and Münzbergová 2008; Knappová et al. 2012), we selected 85 native herbs inhabiting calcareous dry grasslands and abandoned fields in the study region (Table 1, Appendix 1) for which we assembled data on species traits. These target species included both rare and common species in the area. We, however, excluded 25 of them due to missing trait values (Appendix 1). These species are not reported further.

Table 1. – List of 60 target species and their occupancy of suitable habitats in the study area.

Species	Occupancy	Species	Occupancy
Agrimonia eupatoria	0.98	*Leontodon hispidus*	0.79
Anemone sylvestris	0.36	*Leucanthemum vulgare*	0.48
Anthericum ramosum	0.34	*Linum catharticum*	0.87
Anthyllis vulneraria	0.55	*Lotus corniculatus*	0.94
Asperula cynanchica	0.41	*Medicago falcata*	0.72
Asperula tinctoria	0.32	*Melampyrum arvense*	0.27
Aster amellus	0.28	*Melampyrum nemorosum*	0.35
Aster linosyris	0.24	*Onobrychis viciifolia*	0.23
Astragalus glycyphyllos	0.84	*Ononis spinosa*	0.54
Brachypodium pinnatum	0.96	*Origanum vulgare*	0.87
Briza media	0.86	*Peucedanum cervaria*	0.42
Bromus erectus	0.72	*Peucedanum oreoselinum*	0.39
Carex flacca	0.81	*Pimpinella saxifraga*	0.75
Centaurea scabiosa	0.85	*Plantago media*	0.85
Cirsium acaule	0.66	*Potentilla heptaphylla*	0.69
Cirsium eriophorum	0.46	*Primula veris*	0.70
Coronilla varia	0.98	*Prunella grandiflora*	0.68
Dianthus carthusianorum	0.35	*Salvia pratensis*	0.89
Eryngium campestre	0.85	*Sanguisorba minor*	0.94
Filipendula vulgaris	0.33	*Scabiosa ochroleuca*	0.82
Galium verum	0.96	*Sesleria albicans*	0.19
Genista tinctoria	0.56	*Silene vulgaris*	0.65
Geranium sanguineum	0.57	*Solidago virgaurea*	0.34
Hieracium pilosella	0.53	*Stachys recta*	0.62
Hypericum perforatum	0.89	*Tanacetum corymbosum*	0.43
Inula hirta	0.29	*Teucrium chamaedrys*	0.80
Inula salicina	0.87	*Trifolium medium*	0.89
Knautia arvensis	0.98	*Trifolium montanum*	0.51
Koeleria pyramidata	0.45	*Veronica austriaca subsp. teucrium*	0.39
Lathyrus pratensis	0.61	*Viola hirta*	0.43

The nomenclature follows Tutin et al. (1964–1983).

Data collection

To assess habitat occupancy for each target species, we recorded species presence on 371 grasslands in 2009 in the study area. Then we identified habitats suitable for each species. Habitat suitability was estimated by Beals index that expresses the probability of a species presence at a habitat based on the number of joint occurrences with other species of the habitat calculated in the whole dataset (Beals 1984, Münzbergová and Herben 2004). The calculation was performed using presences of all the above 85 species and 35 additional species. The additional species were recorded on the dry grasslands in 2009 to increase sample size for evaluation of habitat suitability and included both species typical of dry grasslands and species representative of habitat deterioration (Appendix 1).

Performances of the models of habitat suitability were evaluated by calculating the area under the receiver operating characteristic curve (AUC, Swets 1988). The receiver operating characteristic curve is obtained by plotting the ratio of correctly predicted presences vs. (1 – the ratio of correctly predicted absences) for varying probability thresholds; AUC values may range from 0 for an inverse model, through 0.5 for a random model, to 1 for a perfect model. After the calculation of Beals index, we identified habitats suitable for particular species. For each model we found the cut-off probability values in which the sum of sensitivity (i.e. the ratio of correctly predicted presences) and specificity (i.e. the ratio of correctly predicted absence) was maximized (Liu et al. 2005). Then we considered all habitats occupied by the species and unoccupied habitats with the probability higher than cut-off level as suitable for given species.

To estimate the age of dry grasslands in the current landscape, we used the information from cadastral maps from 1843 (1:2 880) and special maps from 1954 and 1980s (1980-1986, 1:5 000) which combine topographical and cadastral information. All maps were provided by the Czech Office for Surveying, Mapping and Cadastre. The maps from 1954 were rectified for a previous study (Chýlová and Münzbergová 2008) and used for rectification of the maps from 1843 and 1980s in ArcGIS 9.3 (Environmental Systems Research Institute Inc. 2008). We classified grasslands according their age. Specifically, grasslands were classified as one, two, three or four, if they have been firstly recorded in 2000s, 1980s, 1950s or 1843 time period, respectively. Then we calculated the mean age of occupied and the mean age of all suitable grasslands for each species.

We assembled data on species life-history traits related to dispersal – terminal velocity, rate of epizoochory (i.e. the ability to attach to sheep fur) and rate of endozoochory (i.e. the ability to survive endozoochory), traits related to both dispersal and survival/growth – seed mass and plant height, traits related to survival/growth – seed bank longevity and specific leaf area and traits describing species habitat requirements – Ellenberg indicator values for light and nutrients (Ellenberg 1988, Table 2). Terminal velocity was measured using dropping method (Jongejans and Schippers 1999). Thirty seeds (10 seeds x 3 populations) were released three times from a predefined height. The rate of epizoochory was assessed as the percentage of seeds attached on sheep fur that was four times gently placed on different 100 seeds, lifted and shaken for ten times (Münzbergová 2004). The rate of endozoochory was estimated according to Kleyer et al. (2008). For this experiment, 1350 seeds of each species (150 seeds x 3 sources x 3 repetitions) were used and the percentage of originally viable seeds having survived simulated digestion was calculated. The percentage of viable seeds was assessed by tetrazolium test (Cottrell 1947) using 900 seeds (150 seeds x 3 sources x 2 repetitions). Seed mass was estimated by weighing 150 seeds (10 seeds x 5 sets x 3 populations). Plant height was estimated on 30 flowering individuals (10 individuals x 3 localities). The data on above traits (except the rate of endozoochory) for 30 target species were obtained from Tremlová and Münzbergová (2007). The data on seed bank longevity and specific leaf area were obtained from LEDA database (Kleyer et al. 2008). We calculated the mean value of

records of specific leaf area for each species and the seed bank longevity index according to Bekker et al. (1998). The number of suitable (occupied) habitats and the values of seed mass and plant height were logarithmically transformed before analyses.

Table 2. – List of traits under study, trait units and mean and range of trait values in 60 species used in trait analyses.

Trait	Units	Mean	Range
Light	category 1-9	7.17	5.00-9.00
Nutrients	category 1-9	2.97	1.00-6.00
Plant height	log(m)	-0.41	-1.12-0.02
Rate of endozoochory	%	68.51	0.00-100.00
Rate of epizoochory	%	15.75	0.00-80.00
Seed bank longevity	index	0.13	0.00-0.75
Seed mass	log(mg)	0.02	-3.31-1.43
Specific leaf area	mm^2/mg	20.89	9.31-37.86
Terminal velocity	m/s	2.15	0.26-3.37

Data analysis

We tested the relationship between habitat occupancy and species traits, the age and number of suitable habitats. We also tested the relationship between the age and number of occupied habitats and species traits. All relationships including those among individual traits were investigated by Spearman's rank correlation coefficient in STATISTICA 6.0 (StatSoft, Inc. 2001).

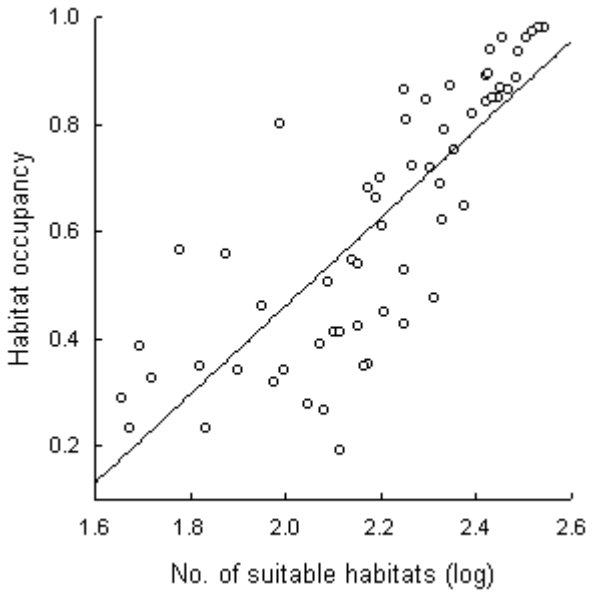

Fig. 1. – Positive effect of the number of suitable habitats on habitat occupancy. The relationship was tested by Spearman's rank correlation analysis (N = 60, r = 0.870, $P < 0.001$).

Results

We identified between 6 and 107 (mean = 51) suitable unoccupied habitats for each of 60 target species. Habitat occupancy ranged from 0.19 to 0.98 (mean = 0.62). AUC values of the models predicting habitat suitability ranged from 0.75 to 0.97 (mean = 0.86), indicating high accuracy of all models. Habitat occupancy was positively significantly correlated ($P < 0.05$) with the number of suitable habitats (Fig. 1) and negatively with habitat age (Fig. 2). Habitat occupancy also positively significantly correlated with seed bank longevity. There was also a marginally significant positive relationship between habitat occupancy and plant height (Table 3). The number of suitable habitats negatively correlated with habitat age (Fig. 3). The number of occupied habitats positively significantly correlated with seed bank longevity. There was also a marginally significant positive relationship between the number of occupied habitats and specific leaf area (Table 3). The age of occupied habitats negatively significantly correlated with nutrients (Fig. 4), seed bank longevity and specific leaf area. A marginally significant negative relationship was also detected for plant height (Table 3). For correlations among species traits see Appendix 2.

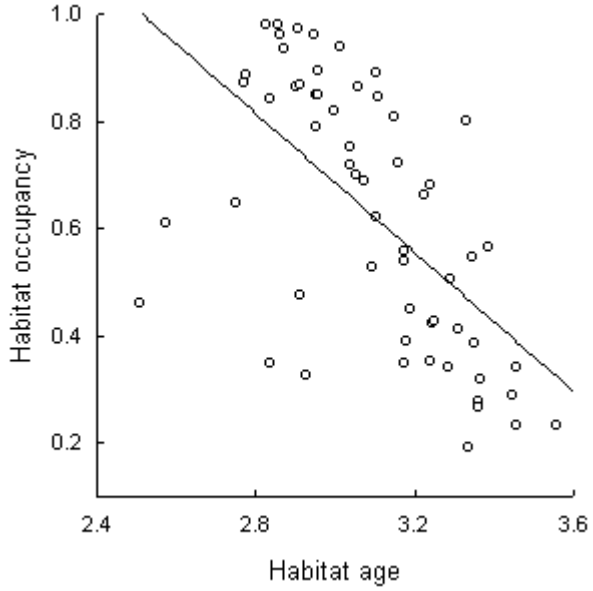

Fig. 2. – Negative effect of the age of suitable habitats on habitat occupancy. The relationship was tested by Spearman's rank correlation analysis (N = 60, r = -0.675, $P < 0.001$).

Table 3. – Effects of life-history traits on habitat occupancy, number and age of occupied habitats as tested by Spearman's rank correlation analyses (N = 60). Significant analyses ($P < 0.05$) are highlighted in **bold**, marginally significant analyses ($P < 0.1$) are in *italic*.

	Habitat occupancy		No. of occupied		Age of occupied	
	r	*P*	r	*P*	r	*P*
Light	0.058	0.658	0.040	0.763	0.070	0.597
Nutrients	0.157	0.230	0.201	0.123	**-0.363**	**0.004**
Plant height	*0.227*	*0.081*	0.187	0.152	*-0.233*	*0.073*
Rate of endozoochory	0.173	0.185	0.105	0.422	-0.089	0.498
Rate of epizoochory	0.096	0.466	0.146	0.266	-0.053	0.688
Seed bank longevity	**0.394**	**0.002**	**0.432**	**0.001**	**-0.459**	**< 0.001**
Seed mass	0.037	0.779	-0.038	0.771	0.092	0.486
Specific leaf area	0.206	0.115	*0.236*	*0.069*	**-0.391**	**0.002**
Terminal velocity	0.088	0.502	0.066	0.617	-0.057	0.667

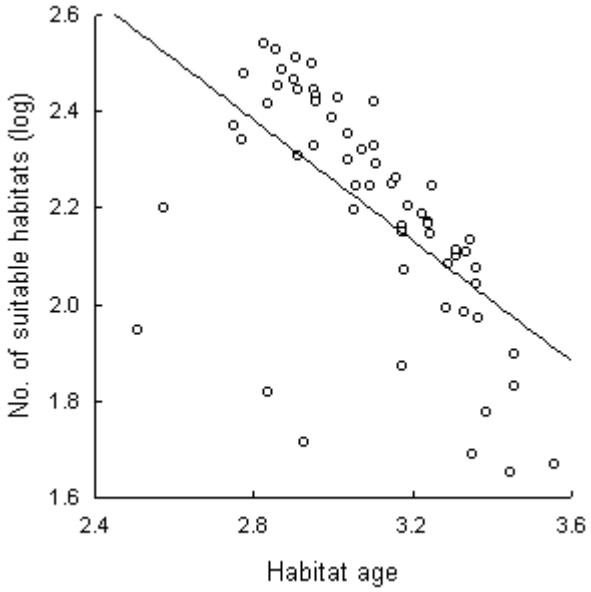

Fig. 3. – Negative relationship between the number and age of suitable habitats. The relationship was tested by Spearman's rank correlation analysis (N = 60, r = -0.706, $P < 0.001$).

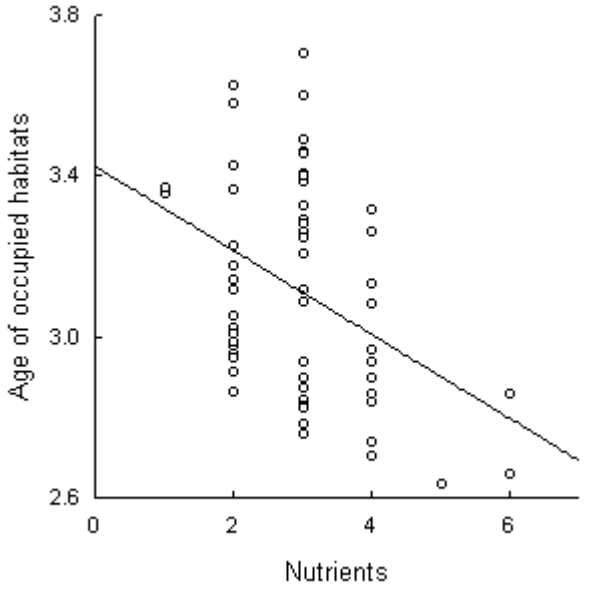

Fig. 4. – Negative relationship between the age of suitable habitats occupied by species and species habitat requirements to nutrients.

Discussion

In the present study, we confirmed a significant relationship between the number and age of suitable habitats and habitat occupancy by the species. Habitat occupancy increased with habitat number and decreased with mean habitat age. Species life-history traits were less important to habitat occupancy than habitat number and age. Among the studied traits, we found seed bank longevity to have a positive effect on habitat occupancy. A positive effect of seed bank longevity has been also found by Piessens et al. (2005) and Tremlová and Münzbergová (2007). In contrast to our findings, Jacquemyn et al. (2003) and Adriaens et al. (2007) have identified no effect of seed bank longevity on habitat occupancy. Jacquemyn et al. (2003) have, however, identified dispersal traits to affect habitat occupancy.

The importance of seed bank longevity indicates that extinctions of local populations are prevented by species recruitment from seed bank rather than by recolonization of habitats. In contrast, higher importance of dispersal traits on habitat occupancy suggests higher colonization rates in such a landscape. We found no effect of dispersal traits on habitat occupancy, although we used multiple vectors instead of single dispersal mode in the present study. We, however, found a marginal positive effect of plant height that is usually considered as dispersal trait (Kleyer et al. 2008). In other studies plant height is often identified to have no effect on habitat occupancy (Eriksson and Jakobsson 1998; Maurer at al. 2003; Piessens et al. 2005; Adriaens et al. 2007, but see Jacquemyn et al. 2003).

Compared to the effect of species traits, the number of habitats and habitat age had much higher effect on habitat occupancy. Habitat number was used as the measure of overall habitat isolation in the landscape as e.g., Levins (1969) or Hanski and Gyllenberg (1993) have hypothesized higher connectivity with higher habitat number which leads to an increase of migration rate. Isolation of individual habitats is a frequent determinant of species distribution (e.g., Eriksson et al. 1995; Bruun 2000; Dupré and Ehrlén 2002; Lindborg et al. 2012). In the present study, however, we also found an effect of the overall isolation of suitable habitats of a species on habitat occupancy and confirmed the previous identical findings of Bastin and Thomas (1999) and Adriaens et al. (2007). Similarly, many studies have found the importance of age of individual habitats to species distribution (e.g., Hérault and Honnay 2005; Endels et al. 2007; De Sanctis 2010). We found a negative effect of habitat age on habitat occupancy. Habitat age, however, decreases with habitat number in the landscape. We found that the number of occupied habitats increases with seed bank longevity, but species that occupied younger habitat more frequently have also higher specific leaf area and species requirements to nutrients. It indicates that younger habitats differ from older habitats in nutrient availability and the level of competition. The divergence in habitat quality of younger and older habitats can be caused by the intensification of agriculture after 1950's (e.g., Bouma et al. 1998; Rabbinge and van Diepen 2000; Václavík and Rogan 2009). Fertilization has been found to have a negative effect on the occurrence of some grassland species (e.g., Eriksson et al. 1995).Therefore we suppose that younger habitats are suitable for generalists, i.e. the species with wider range of habitat suitability as both old and young habitats are suitable for these species. By contrast, specialists are often restricted to old habitats which leads to low total number of their suitable habitats. Although we identified seed bank longevity as the only important trait for habitat occupancy, requirements to nutrients and specific leaf area affect habitat occupancy indirectly through the number of habitats suitable for given species.

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Appendix 1. – List of 25 excluded species with missing life-history trait values and 35 additional species used for estimation of habitat suitability.

Missing traits	Used for habitat suitability
Astragalus cicer	*Achillea millefolium*
Bupleurum falcatum	*Arrhenatherum elatius*
Campanula glomerata	*Artemisia campestris*
Campanula rotundifolia	*Calamagrostis epigejos*
Carex tomentosa	*Carduus acanthoides*
Carlina vulgaris	*Carex humilis*
Centaurea jacea	*Centaurea rhenana*
Cirsium pannonicum	*Dactylis glomerata*
Coronilla vaginalis	*Daucus carota*
Falcaria vulgaris	*Echinops sphaerocephalus*
Festuca rupicola	*Echium vulgare*
Globularia elongata	*Elymus hispidus*
Helianthemum nummularium subsp. grandiflorum	*Elymus repens*
Laserpitium latifolium	*Euphorbia cyparissias*
Linum flavum	*Euphrasia rostkoviana*
Linum tenuifolium	*Fragaria viridis*
Plantago lanceolata	*Galium album*
Prunella vulgaris	*Galium boreale*
Salvia nemorosa	*Gentiana cruciata*
Salvia verticillata	*Gymnadenia conopsea*
Scabiosa canescens	*Inula conyza*
Scorzonera hispanica	*Listera ovata*
Seseli hippomarathrum	*Medicago sativa*
Vicia cracca	*Medicago varia*
Vicia sepium	*Melampyrum cristatum*
	Nonea pulla
	Picris hieracioides
	Platanthera bifolia
	Polygala vulgaris
	Potentilla arenaria
	Pulsatilla pratensis subsp. bohemica
	Thesium linophyllon
	Thymus praecox
	Tragopogon pratensis subsp. orientalis
	Verbascum lychnitis

Nomenclature follows Tutin et al. (1964-1983).

Appendix 2. – Spearman's rank correlation coefficients of traits under study. Significant correlations ($P < 0.05$) are highlighted in **bold**.

	Light	Nutrients	Plant height	Rate of endo.	Rate of epi.	Seed bank longevity	Seed mass	SLA
Nutrients	-0.19							
Plant height	0.10	0.22						
Rate of endo.	0.16	0.09	0.08					
Rate of epi.	**0.29**	0.02	-0.03	-0.17				
Seed bank longevity	0.01	0.22	0.02	0.16	-0.14			
Seed mass	0.06	0.15	**0.34**	**0.31**	-0.15	-0.05		
SLA	-0.25	**0.28**	-0.04	0.14	-0.16	**0.28**	0.02	
Terminal velocity	**-0.28**	0.18	0.17	**0.31**	**-0.51**	0.07	**0.48**	0.25

Abbreviations: endo = endozoochory; epi = epizoochory; SLA = Specific leaf area.

Chapter 5

The effects of large herbivores on the landscape dynamics of a perennial herb

Lucie Hemrová, Zita Červenková and Zuzana Münzbergová

Abstract

Models assessing the prospects of plant species at the landscape level often focus primarily on the relationship between species dynamics and landscape structure. However, the short-term prospects of species with slow responses to landscape changes depend on the factors affecting local population dynamics. In this study it is hypothesized that large herbivores may be a major factor affecting the short-term prospects of slow-responding species in the European landscape, because large herbivores have increased in number in this region in recent decades and can strongly influence local population dynamics. The impact of browsing by large herbivores was simulated on the landscape-level dynamics of the dry grassland perennial polycarpic herb *Scorzonera hispanica*. A dynamic, spatially explicit model was used that incorporated information on the location of patches suitable for *S. hispanica*, local population dynamics (matrices including the impact of large herbivores), initial population sizes and dispersal rate of the species. Simulations were performed relating to the prospects of *S. hispanica* over the next 30 years under different rates of herbivory (browsing intensity) and varying frequencies of population destruction (e.g., by human activity). Although a high rate of herbivory was detected in most populations of *S. hispanica*, current landscape-level dynamics of *S. hispanica* were approximately in equilibrium. A decline or increase of over 20% in the herbivory rate promoted rapid expansion or decline of *S. hispanica*, respectively. This effect was much stronger in the presence of population destruction. Browsing by large herbivores can have a dramatic effect on the landscape dynamics of plant species. Changes in the density of large herbivores and the probability of population destruction should be incorporated into models predicting species abundance and distribution.

Keywords: *Bromion erecti*, grazing, landscape-level modelling, large ungulates, roe deer.

Introduction

Rapid changes in the landscape in recent years have resulted in increased levels of habitat fragmentation for many plant species. These changes have provoked discussion about the prospects of such species in the future agricultural landscape (e.g., Saunders et al. 1991; Collinge 1996; Bastin and Thomas 1999; Lindborg and Eriksson 2004). Several authors have emphasized the importance of describing species dynamics at the landscape level to estimate the future prospects of species (e.g., Eriksson 1996; Husband and Barrett 1996; Bastin and Thomas 1999; Hanski 1999).

Modelling studies that simulate species dynamics at the landscape level focus primarily on the impact of changes in landscape structure (e.g., With et al. 1997; Hanski and Ovaskainen 2000; Herben et al. 2006; Alados et al. 2009). However, for species that respond slowly to landscape change (e.g., long-lived species with limited dispersal; e.g., Mildén et al. 2006), changes in landscape structure may not be the most important factor influencing species dynamics. Instead, the factors that affect local population dynamics are probably crucial in influencing landscape-level species dynamics over the short term.

Browsing by large herbivores is one of the most important factors affecting local population dynamics of plant species (e.g., Bergelson and Crawley 1992; Augustine and Frelich 1998; Russell et al. 2001; Rooney and Waller 2003). Browsing can strongly influence local population dynamics by affecting the components of the plant life cycle, such as seedling survival (Paige and Whitham 1987; Knight et al. 2008), plant seed production (Knight et al. 2008; Ehrlén and Münzbergová 2009; Lin and Galloway 2009) and the probability of flowering in the next season (Knight et al. 2008; Ehrlén and Münzbergová 2009). In addition to these negative effects, large herbivores can have positive effects on long-distance dispersal (reviewed in Nathan et al. 2008) and thus on species colonization. Large herbivores can also positively affect plant population growth rate by enhancing seedling recruitment (reviewed by Maron and Crone 2006). The number of large herbivores, such as roe deer, has been increasing in the agricultural European landscape in recent decades (Meriggi et al. 2008). Increased herbivory and dispersal rates due to a higher number of large herbivores can have both negative and positive effects on the prospects of plant species in the landscape.

In the present study, we estimated the prospects of a grassland polycarpic perennial herb, *Scorzonera hispanica*, at the landscape level, incorporating the effect of browsing by large herbivores on the species dynamics. Specifically, the aims of the present study were to model the landscape dynamics of *S. hispanica* in northern Bohemia (Czech Republic) and to simulate the prospects of this species in the near future. Although *S. hispanica* is considered endangered in the Czech Republic, it is common in the study area. In the area, the species occurs on clearly delimited patches. Some of these patches are suitable but unoccupied, as identified by means of a sowing experiment indicating that *S. hispanica* is dispersal-limited (Münzbergová 2004). Chýlová and Münzbergová (2008) demonstrated that this species prevails in grasslands established for at least 60 years, indicating that the dynamics of the species are quite slow. *S. hispanica* does not form a permanent seed bank (Münzbergová 2004). Recolonization is thus only possible by means of long-distance dispersal. All the above properties indicate that *S. hispanica* fulfils the criteria for possessing metapopulation dynamics (Freckleton and Watkinson 2002). Information on landscape-level dynamics of this species can thus be generalized to other species fulfilling the same criteria with slow response to landscape changes. The identified patterns could thus be generalized for many grassland and forest-understorey long-lived perennial herbs.

Browsing by large herbivores has been observed in most *S. hispanica* populations. We have also observed the destruction of habitats of *S. hispanica* within the study region, due primarily to ploughing, the construction of solar power stations or rooting by wild boars. Therefore, in the present study, we evaluated the effects of both browsing by large

herbivores and population destruction on the landscape-level dynamics of *S. hispanica*, as they both may influence its future prospects.

To understand the future dynamics of *S. hispanica* in the landscape, we asked the following questions: (i) What are the future prospects of *S. hispanica* in the current landscape and under the current rate of herbivory (i.e. browsing by large herbivores)? (ii) What is the effect of herbivory on the future prospects of *S. hispanica*? (iii) What are the combined effects of herbivory and population destruction on the prospects of *S. hispanica*?

To answer these questions, we parameterized a model of landscape dynamics for *S. hispanica* based on available information on the distribution of suitable habitats, the local population dynamics (including the current rate of herbivory and the risk of population destruction), dispersal ability and current population sizes. We then simulated the prospects of the species after 30 years under a wide range of herbivory rates and with different levels of risk of population destruction. We assumed that the landscape would not change dramatically over such a short period and that the response to landscape changes would be slow. Under these assumptions, browsing by large herbivores is expected to be the primary factor influencing the prospects of *S. hispanica*. Model credibility was tested by performing sensitivity analyses of the model parameters.

Methods

Study species and study area

Scorzonera hispanica L. (Asteraceae) is a rare, allogamous, polycarpic perennial herb inhabiting the dry grasslands of central and southern Europe. It has a single rosette and a single flowering stalk with one to seven yellow flowerheads. It is occasionally cultivated for its edible rootstock (Chater 1976). The fruits of *S. hispanica* are achenes with a pappus. The presence of the pappus enables dispersal by wind and exozoochory. The species does not form a persistent seed bank; the seeds, which do not germinate, decompose within 2 years (Münzbergová 2004).

In the Czech Republic, *S. hispanica* is a native species and is considered endangered. It occurs in central and northern Bohemia and in southern Moravia, occupying calcareous dry grasslands (alliance *Bromion erecti* of Ellenberg 1988). To model the prospects of *S. hispanica* under different rates of herbivory and population destruction, we focused on a typical agricultural landscape with a common occurrence of both *S. hispanica* and large herbivores. All study populations are browsed by ungulates. No other type of herbivory has been observed. Roe deer, mouflon and wild boar are very common in the landscape, whereas fallow deer and red deer occur only rarely (Municipality Litoměřice, Department of Environment). Only browsing by roe deer, common herbivores of numerous plant species in both grassland and forest-understorey (e.g., Gill et al. 1996; Jepsen and Topping 2004; Hewison et al. 2007), has been observed in *S. hispanica* populations. However, we consider that the other large herbivores in the landscape can also occasionally browse *S. hispanica*.

The study area (4.39 × 4.39 km) was situated in northern Bohemia in the Czech Republic (50°33′26″N, 14°12′45″E, to 50°31′21″N, 14°17′3″E). Calcareous dry grasslands are typical of the landscape. These grasslands form distinct patches surrounded by shrubs and large agricultural fields. These formerly maintained grasslands are now unmanaged and therefore experience very slow succession of shrubs and trees. Population sizes range from three to 2500 flowering individuals. Genetic variability in the field is high; Nei's genetic diversity values range from 0.04 to 0.32, indicating that all populations are genetically variable (Münzbergová and Plačková 2010). Large herbivores favour the flowering stalks of *S. hispanica*. Our long-term field observations indicate that the

flowering stalks of *S. hispanica* are browsed extensively without signs of leaf herbivory on the browsed individuals or on the surrounding vegetation (Z. Münzbergová, pers. obs.).

Field data collection

All dry grassland patches (73 in total, from 48.7 to 214 396.3 m^2) in the study area were located within the region studied by Chýlová and Münzbergová (2008). In their study, a digital map of dry grassland patches was created and the presence of *S. hispanica* and 65 other species (Supplementary Data Table S1) were recorded at each patch. We added data from 12 populations (patches) of *S. hispanica* outside the study area to the present dataset to increase sample size. All external patches were ≤ 30 km from the study area and ranged in size from 882.9 to 62 365.9 m^2. The external patches all hosted the same dry grassland vegetation (i.e. *Bromion erecti*, Ellenberg 1988) as the patches within the study area. At each external patch, we recorded the presence of the 65 selected species of dry grassland vegetation. We counted the number of flowering *S. hispanica* individuals at all patches. We surveyed 85 patches of dry grassland, 35 of which hosted *S. hispanica*. The external patches were used to improve the predictive power of models of patch suitability and of the herbivory rate at each patch. External patches were not used to simulate the prospects of *S. hispanica* in the study area.

To model the impact of large herbivores on *S. hispanica* landscape-level dynamics, we incorporated the effect of herbivory on performance of *S. hispanica* into transition matrix models of the local population dynamics of the species. We used a set of eight transition matrices containing three size classes (seedling, large vegetative and flowering individuals) to simulate local population dynamics. These eight matrices were constructed for a previous study (Münzbergová 2006) and included data collected between 2001 and 2004 in three populations over three transition intervals (population nos. 16, 18 and 20 in Table 1 in Münzbergová 2006). Population size ranged between 1632 and 2464 individuals, with at least 150 individuals marked in each population; see Münzbergová (2006) for additional details. Two populations (nos. 16 and 20) are found within the present study area; the third is nearby and occurs in the same type of habitat. This latter population is among the 12 external populations described above. We considered these populations to be representative as they contain a sufficient number of individuals for studying population dynamics and exhibit habitat conditions typical of other populations in the area. Two populations have been largely stable over the last 10 years. However, all marked plants in the third population were destroyed by wild boars during the last transition period; therefore, no transition matrix could be built from these data.

The plants used for matrix construction experienced browsing by large herbivores; however, browsing intensity was not quantified. It was thus necessary to identify those transitions within the matrices that were affected by herbivory and to replace these transitions by probabilities with a quantified rate of herbivory. Z. Červenková (unpubl. res.) found that only flowering stalks were browsed; there was very little herbivore damage to vegetative plants. Z. Červenková (unpubl. res.) also estimated the impact of large herbivores on performance of flowering *S. hispanica* in a field experiment (Supplementary Data Appendix S1). Specifically, she protected selected plants from browsing using cages and compared the performance of intact and browsed plants. She found that herbivory decreased the seed production and the production of clones by flowering plants. No other impact of herbivory was found. In addition, Münzbergová (2004) demonstrated that the recruitment and survival of seedlings and adult *S. hispanica* plants are not affected by the above-ground biomass at the localities. Seedling recruitment and survival are also unaffected by the presence of open spaces in the vegetation (Z. Münzbergová, unpubl. res.). These findings suggest that neither biomass removal nor an increase in canopy openness due to herbivory affect the reproductive success of *S. hispanica*. We therefore

focused only on the impact of large herbivores on flowering individuals in the present study.

To estimate the rate of herbivory of flowering stalks, we collected data on intensity of browsing from 21 *S. hispanica* populations of varying size in 2009 and 2010. Ten populations were within the study area, and 11 were external. In all 21 populations, we recorded the total number of browsed and intact flowering plants. In populations comprising fewer than 150 flowering individuals, we recorded browsing data from all flowering plants present. In the larger populations, we collected data from approx. 150 flowering plants sampled along randomly selected transects. The study was conducted at the end of the flowering period (mid-July), when herbivory on *S. hispanica* ends but faded flowerheads are still present on the stalks (the herbivores browse flowering, not mature, flowerheads).

Habitat characteristics

To estimate the suitability of individual grassland patches for *S. hispanica* and to identify the factors affecting the herbivory rate, we recorded data on 26 characteristics at each patch. These included both local habitat characteristics and characteristics describing landscape structure (Table 1), e.g., the location of individual patches in the landscape. To obtain data on the local habitat characteristics, we first constructed digital elevation models (DEMs) with a 5-m grid size. DEMs were derived from digital contours (1:10 000, 2-m vertical distance between contours) provided by the Czech Office for Surveying, Mapping and Cadastre. DEMs were constructed for the entire study area and the 12 external patches in ArcGIS 9.2 (Environmental Systems Research Institute Inc., Redlands, CA, USA). Based on these models, we created grids of slopes and potential direct solar irradiation (PDSI) for the 21st day of the month from December to June using ArcGIS 9.2, and created grids of topographic wetness index (TWI) using SAGA GIS 2.0.4. (SAGA User Group Association, Hamburg, Germany). For each patch, we then calculated the logarithm of the total area, the mean values of slopes, PDSI (from December to June) and TWI (Table 1).

Other local habitat characteristics were calculated using the presence of the 65 selected species from our species list (Supplementary Data Table S1). First, we calculated the Beals index, which expresses the probability of a species presence at a patch using the number of joint occurrences with other species (Beals 1984; Münzbergová and Herben 2004). We used the presence of all plant species from the species list in all patches for this calculation. Second, we calculated Ellenberg indicator values of light, temperature, moisture, nutrients, soil reaction and continentality (Ellenberg 1988; Table 1) for each patch using all species recorded at the patch.

To obtain the parameters describing landscape structure around the patches, we calculated the nearest distance between each patch and shrubs, forests, roads and villages; we also recorded the amount of shrub and tree cover surrounding each patch (Table 1). We used digital maps of shrubs, forests, roads and villages for these calculations. The digital map of shrubs and forests based on NATURA 2000 mapping was provided by the Agency for Nature Conservation and Landscape Protection of the Czech Republic. The digital maps of roads and villages were created by combining information on the latest online cadastral and orthophotomaps provided by the Czech Office for Surveying, Mapping and Cadastre and by the Czech Environmental Information Agency, respectively. Using the digital maps, we first calculated the areas of both shrubs and forests within both a 500-m and 1-km radius of each patch. We then calculated the distance between each focal patch and (1) the nearest shrub, (2) the nearest forest, (3) the nearest road and (4) the nearest village (Table 1). Distances were calculated between centre points of each patch to the boundaries of these objects using ArcGIS.

Table 1. – List of recorded habitat characteristics used to develop predictive models of (1) patch suitability for *S. hispanica* ($P < 0.001$, $F = 16.57$, d.f. error = 73, $R^2 = 0.75$) and (2) herbivory rates at patches ($P < 0.001$, $F = 88.61$, d.f. error = 15, $R^2 = 0.78$). +/– represents the positive/negative effect of characteristics included in the final model (significant values at $P < 0.05$ are in bold type), n indicates characteristics not included in the model and x indicates characteristics excluded from the test.

	Patch suitability		Herbivory rate	
		R^2		R^2
Local habitat characteristics				
Area		n	-	0.022
Slope		n		x
TWI	-	**0.018**	-	0.004
PDSI				
December		n		x
January		n		x
February	-	**0.143**		x
March	+	**0.147**		x
April	-	**0.128**		x
May		n		x
June	+	**0.113**		n
Beals index	+	**0.483**		x
Ellenberg indicator values				
Light	-	**0.042**		x
Moisture		n		x
Soil reaction	+	**0.032**		x
Nutrients	+	**0.146**		n
Temperature	+	**0.048**		x
Continentality	+	**0.045**		x
Landscape structure				
Forest 1 km		x		n
Shrub 1 km		x	-	**0.359**
Forest 0.5 km		x		n
Shrub 0.5 km		x		n
Nearest forest		x		n
Nearest shrub		x		n
Nearest village		x	+	0.033
Nearest road		x	-	0.025
Population size		x		n

Data analysis

To estimate patch suitability, we tested for the effects of habitat characteristics on the occurrence of *S. hispanica*. We used a generalized linear model (GLM) with a binomial distribution of the dependent variable (presence/absence of *S. hispanica*) in this test. We excluded data on landscape structure from our independent variables, as they related to patch availability not to patch suitability. We used data from all 85 patches, 35 of which hosted *S. hispanica*. To simplify the model (correlation matrix in Supplementary Data Table S2), we used step-wise both-direction regression starting with the maximal model. We identified those habitat characteristics that best explained *S. hispanica* presence using the Akaike information criterion (AIC; Crawley 2002). Based on this model, we calculated the probability of *S. hispanica* presence at each patch (Crawley 2002). These probabilities were used to identify suitable unoccupied patches for *S. hispanica* (see '*Simulation plane*' below).

Our investigation of the factors determining *S. hispanica* herbivory rate involved a small number of observations (21 populations). Therefore, we primarily selected those landscape and habitat characteristics expected to influence herbivory rate (Table 1).

Specifically, we used the area of forests and shrubs surrounding each patch within a radius of 0.5 and 1 km and the distances to the nearest forest, shrub, village and road as possible factors influencing the behaviour of large herbivores and the subsequent herbivory rate (e.g., Welch et al. 1990; Tufto et al. 1996; Hewison et al. 2001; Nilsen et al. 2004; Coulon et al. 2008). We also evaluated factors related to site vegetation, including *S. hispanica* population size, TWI, PDSI in June (i.e. in the growing season), the Ellenberg indicator value for nutrients and the Beals index (Table 1).

To identify the characteristics influencing herbivory rate, we used the mean rate of herbivory in 2009 and 2010 as the dependent variable (herbivory rates did not differ significantly between years, data not shown). The total numbers of browsed and intact plants over both years were used as the dependent variables with a binomial distribution in a GLM. As we had three similar measures of the impact of forest and shrubs, we made three partial tests, using: (1) the amount of cover of shrubs and forests within 1 km, (2) the amount of cover within 500 m and (3) the proximity of the nearest shrub and forest. In each test, we performed step-wise, bothdirection logistic regression (using AIC; Crawley 2002) starting with the maximal model. We then chose the best model (i.e. the model with the lowest number of independent variables and the highest explanatory power). Using the selected model, we calculated the predicted rate of herbivory (Crawley 2002) at each patch in the area. All analyses were performed in S-Plus Professional Release 2 (MathSoft, Inc., Seattle, WA, USA).

Model description and estimation of model parameters

To simulate the dynamics of *S. hispanica* in the landscape, we used a dynamic, spatially explicit landscape-level model presented in previous studies by Münzbergová et al. (2005), Herben et al. (2006) and Mildén et al. (2006), following similar methods. This model does not assume equilibrium between species extinction and colonization; this assumption is important because disequilibrium species dynamics have often been observed in long-lived species following rapid changes in landscape structure (e.g., Matlack 1994; Eriksson 1996; Brunet et al. 2000; Lindborg and Eriksson 2004; Herben et al. 2006). The model uploads (1) the information on location and size of suitable patches (habitats) for a species, (2) the initial habitat occupancy including local population sizes, (3) a set of matrices simulating local population dynamics and (4) the coefficients of dispersal curves (exponential and/or hyperbolic functions) and proportion of seeds dispersed independent of distance (for model details see Supplementary Data Appendix S2).

Simulation plane. Suitable patches were identified on the grid (5-m cell resolution) by the probabilities of *S. hispanica* presence. Patches were classified as either suitable or unsuitable by finding the lowest calculated probability in the set of patches that, in the actual study area, host *S. hispanica*. We then considered all patches of the same or higher probability to be suitable for the species assuming that *S. hispanica* occurred on suitable patches only. We also found probability thresholds using methods recommended by Liu et al. (2005). As the threshold we took either the prevalence of the model-building data or the average predicted probability of the model-building data. Compared with the original threshold, these two thresholds identically identified three more unoccupied patches as unsuitable for *S. hispanica*. The lower number of suitable unoccupied patches was then used to estimate the sensitivity of the model to landscape structure. However, the changed model provided similar results to the original and is not discussed further.

Local population dynamics, herbivory and population destruction. Suitable patches were classified according to the predicted herbivory rate into 11 categories of habitat quality, corresponding to proportions of browsing of 0–100% at 10% step intervals. We then used

the eight available transition matrices of the Münzbergová (2006) study to build 88 additional matrices (11 from each matrix). Each set of eight matrices included the rate of herbivory corresponding to specific herbivory rate (ranging from 0–100 %, 10% step intervals). Specifically, in each of the eight matrices, we substituted those transitions significantly affected by browsing with the weighted mean of transition values in browsed and intact plants found by Z. Červenková (Supplementary Data Appendix S1). The set of the eight matrices with specific rate of herbivory was assigned to each patch according its predicted herbivory rate.

Ploughing, construction and rooting by wild boars occasionally occurring in the landscape can cause destruction to varying extents. Therefore, we included the probability of population destruction in the model. We had no reliable estimates of the probability and extent of population destruction. However, we assumed that some individuals could survive during the destruction. We thus used the 88 transition matrices described above and decreased all transition probabilities by 90% to obtain a set of 88 modified matrices. In this way 90% of all individuals that would have survived into the next year did not survive. Modified matrices were used at various frequencies to original ones. In all simulations, except those modelling the impact of population destruction on prospects of *S. hispanica*, we used a frequency of one disturbance matrix per 29 original matrices, i.e. one population destruction per 30 years per population. This proportion was chosen based on the observation of Münzbergová (2006) and our subsequent monitoring of the populations in the area.

Initial population size. The numbers of seedlings and vegetative individuals at each patch were calculated from the numbers of flowering individuals (counted in the field) according to the mean stable stage distribution occurring under a specific rate of herbivory. However, stable stage distributions are reached only in populations with stable local population dynamics. To estimate the sensitivity of the model to this assumption, we used half the numbers of seedlings and vegetative individuals calculated from the stable stage distribution. The results of this alternative model were, however, very similar to the original and are therefore not reported further.

Density-dependence. To simulate density-dependence, we estimated the maximum population density at any patch, based on the number of *S. hispanica* individuals at each patch and patch size. The calculated maximum was 0.97 individuals m^{-2}. Based on our field experience, we assumed that the maximum density of seedlings was four times higher than the maximum density of vegetative or flowering individuals. Thus, seedlings had one-quarter the competitive effect of flowering and vegetative individuals. We also performed a sensitivity analysis of this parameter, using 0.97/3 or 0.97 × 3 individuals m^{-2} during the simulations.

Dispersal. We assumed no incoming diaspores to the simulation plane, as the study area was somewhat isolated from other *S. hispanica* populations (the nearest flowering population was 4.3 km from the area border). Therefore, low numbers of incoming diaspores could be expected. Outgoing diaspores during the simulations were considered to be lost. *S. hispanica* was expected to disperse by wind and exozoochory. Wind dispersal was modelled as distance-dependent using a negative normalized exponential function (Münzbergová et al. 2010):

$$y = \alpha * \exp(-\alpha * x),$$

where y is the proportion of seeds dispersed to distance x and α is the coefficient indicating the shape of the curve. We used the exponential model due to its simple normalization,

which was necessary for calculation of the dispersal coefficient of the curve using data on wind speed and species traits. Commonly used dispersal models (including the exponential) tend to underestimated long-distance dispersal (e.g., Bullock and Clarke 2000; Nathan et al. 2002). We thus modelled *S. hispanica* landscape dynamics under several scenarios of dispersal ability (including different exponential curves). We then checked whether higher/lower dispersal ability influenced the results of the model. Specifically, dispersal coefficient a was calculated as 1/*D*, where *D* is the mean dispersal distance of the seeds calculated from the formula (e.g., Augspurger 1986; Soons and Heil 2002; Tremlová and Münzbergová 2007)

$$D = wh/t,$$

where *w* is wind speed ($m.s^{-1}$), *h* is the height of inflorescence (m) and *t* is the terminal velocity ($m.s^{-1}$) of the fruits. For the measure of wind speed *w*, we used the maximum daily mean wind speed over June and July, as detected by the Czech Hydrometeorological Institute at the nearby meteorological station in Doksany from 2005 to 2010 (9.6 $m.s^{-1}$). Wind speeds detected during this period ranged from 3.4 to 32.6 $m.s^{-1}$. Inflorescence height *h* was estimated from 2007 data on 70 plants in seven populations (ten from each population, range = 0.17–0.83 m, mean = 0.47 m). We used the mean terminal velocity of *S. hispanica* fruits (1.78 $m.s^{-1}$) estimated by Münzbergová (2004). Due to the lack of data on the range of terminal velocities, we chose a range of 1.78 $m.s^{-1}$ ± 33%. Based on the ranges of all parameters (*w*, *h* and *t*), we calculated a dispersal distance range of 0.24–22.80 m and a mean dispersal distance of 2.51 m. In the simulations, we used the mean dispersal distance. Minimum and maximum dispersal distances were used to perform sensitivity analyses of the dispersal parameter.

We did not have an estimate of the proportion of seed dispersed via animal fur in the field as obtaining realistic estimates of such a value is difficult (Nathan et al. 2008). Therefore, we assumed that only 0.1% of all seeds were dispersed by exozoochory and by rare events (see also Münzbergová et al. 2005). As herbivores attack individuals during flowering and not when the seeds are mature, the proportion of damaged flowerheads with mature seeds is very low. Therefore, endozoochory was not considered in our simulations. Exozoochory was assumed to affect primarily the long-distance dispersal of *S. hispanica*. It was modelled as independent of distance. Although this assumption seems to be unrealistic, it was used in previous studies (e.g., Münzbergová et al. 2005; Mildén et al. 2006). In our study, dispersal was modelled within small study area. This does not suggest that dispersal is independent of distance at any scale, but rather that the animals can easily cross the whole model landscape within a short period of time. The sensitivity analysis of distance-independent dispersal was performed using 1% and 0.01% of the dispersed seeds.

Simulations

All forecasts were run for 30 steps (30 simulation years), with each forecast replicated 100 times. We ran the simulations for up to 30 years, assuming that the landscape would not change dramatically over such a period. However, running the model for 100 years provided qualitatively very similar results, with the time frame having no impact on our conclusions (data not shown).

We estimated the impacts of the range of the model parameters on the prospects of *S. hispanica* under different herbivory rates. First, we simulated the prospects of the species under the current rate of herbivory predicted for individual patches in the area. We then simulated a gradual (at 10% intervals) decrease (or increase) in the predicted rate of herbivory over the entire study area, until the herbivory rate (i.e. the number of browsed

flowering individuals) decreased to 0% (or increased to 100 %) in all patches. In this way, we obtained 17 different simulations of different herbivory rates.

To assess the effect of the frequency of population destruction on the prospects of *S. hispanica*, we simulated different rates of population destruction under different herbivory rates. The frequencies of population destruction ranged between 0.5 and 10 disturbances per population per 30 years. We obtained 11 different simulations of the impact of disturbance regime on the prospects of *S. hispanica* under 17 different rates of herbivory.

Results

Determinants of patch suitability and herbivory rate

The step-wise regression identified 11 of 17 local habitat characteristics as significant predictors of the presence of *S. hispanica* in a patch (Table 1). These include PDSI in various months, the Beals index and the Ellenberg indicator values. Based on the model, we identified 31 patches as suitable for *S. hispanica*, eight of which were unoccupied. Five of 12 landscape and local habitat characteristics were selected in the step-wise regression as significant predictors of the rate of herbivory in a patch (Table 1): patch area, TWI, shrub cover within 1 km of the patch, proximity of the nearest village and proximity of the nearest road. The predicted rate of herbivory ranged from 40 to 100% (mean 77 %, median 80 %) among single patches.

Impact of herbivores at the landscape scale

Our simulations revealed a strong effect of large herbivores on the long-term prospects of *S. hispanica*. Simulation using the predicted herbivory rate showed an equilibrium in the number of *S. hispanica* individuals in the area (mean population size after 30 years = 86 371, s.d. = 23 632; initial population size = 78 462, Fig. 1A). Under high rates of herbivory, *S. hispanica* tended to go extinct; under low rates, population size increased substantially. Similarly, herbivory rates had a negative effect on patch occupancy (Fig. 1B). Using the current rate of herbivory in our simulations, the patches hosting small populations experienced higher turnover of patch occupancy than the patches hosting large populations (Fig. 2).

Sensitivity analyses of dispersal parameters (Supplementary Data Figs S1a,b and S2a,b) revealed an effect of both wind dispersal and exozoochory on the number of occupied patches under low rates of herbivory at the end of the simulations (Supplementary Data Fig. S2a,b). Sensitivity analysis of maximum population density (Supplementary Data Figs S1c and S2c) showed a strong effect on the total number of individuals under low rates of herbivory (Supplementary Data Fig. S1c). In both cases, parameter effects disappeared under high rates of herbivory, which indicates that increased herbivory reduces the positive effects of longer dispersal rates and higher carrying capacity (Supplementary Data Figs S1 and S2).

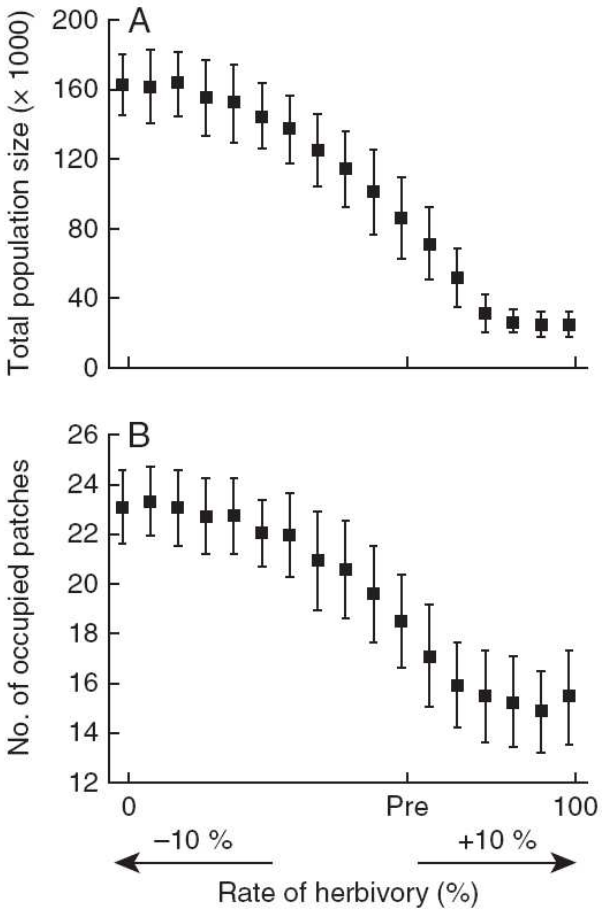

Fig. 1. – The negative effects of increasing herbivory rates on (A) the total number of *S. hispanica* individuals and (B) the number of patches occupied by *S. hispanica* at the end of simulations. The mean ± s.d. (obtained from 100 repetitions of each simulation) are shown for all simulations. 'Pre' shows the current rate of herbivory predicted in each patch in the area. The rate of herbivory was increased or decreased incrementally by 10% for all patches until it reached 100% or 0 %, respectively.

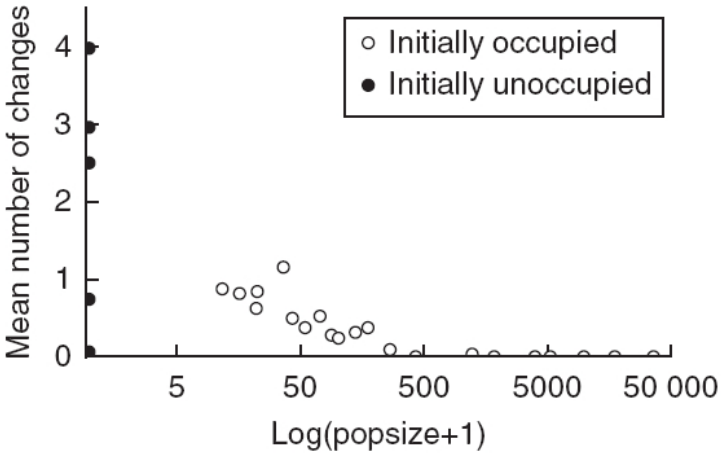

Fig. 2. – The relationship between initial population size and tuzrnover of patch occupancy during the simulation, using the predicted current rate of herbivory in the area. The mean number of changes (i.e. extinctions and colonizations) over all 100 repetitions of the simulation for each of 31 suitable patches in the area is plotted.

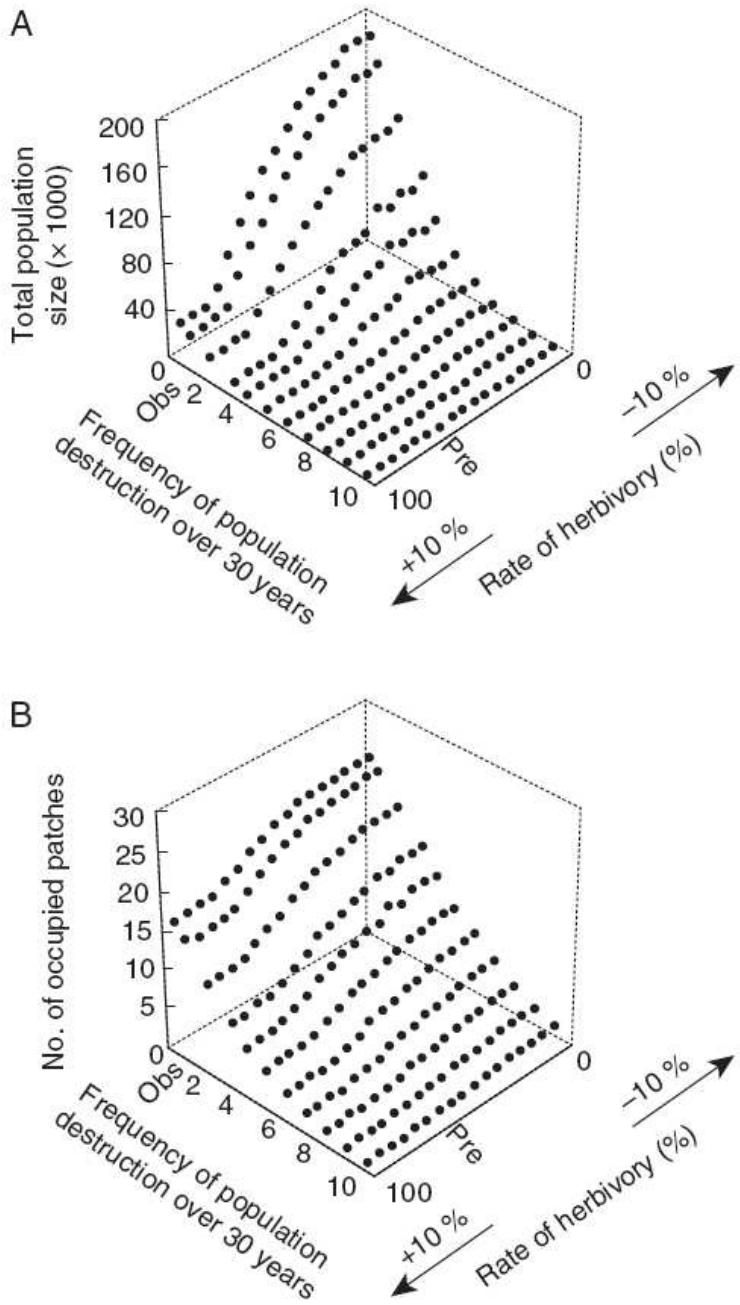

Fig. 3. – The negative effects of increasing herbivory rates and the frequency of disturbances in the area on (A) the total number of *S. hispanica* individuals and (B) the number of patches occupied by the species at the end of simulations. 'Pre' shows the current rate of herbivory predicted in each patch in the area. The rate of herbivory was increased or decreased incrementally by 10% for all patches until it reached 100% or 0 %, respectively. 'Obs' shows the observed frequency of population destruction in the area (one per population per 30 years).

Our simulations also revealed that not only herbivory rates but also frequencies of population destruction had a strong effect on the landscape-level dynamics of *S. hispanica*. Rates of population destruction higher than those observed in the field (i.e. one per 30 years in a patch) led to a considerable decrease in the number of individuals and the number of occupied patches in the area (Fig. 3). The pattern was observed for all rates of herbivory. A rapid decline of the number of *S. hispanica* individuals was observed when high frequencies of population destruction were combined with high rates of herbivory.

Discussion

In this study, we demonstrated the importance of browsing by large herbivores on the landscape-level dynamics of *S. hispanica*. Despite the negative effects of browsing on the performance of *S. hispanica*, the landscape-level dynamics of *S. hispanica* are currently at equilibrium. The future prospects of this species, however, depend on the prospects of large populations. We also found that the potential effects of large herbivores on landscape-level dynamics may be considerable. Simulated declines or increases in the rate of herbivory throughout the landscape by more than 20% often led to the rapid expansion or decline of *S. hispanica*. A similar effect of herbivory rate was observed in the sensitivity analyses of various model parameters. These findings indicate that herbivores can be among the major drivers of landscape dynamics of long-lived perennial herbs.

The results of the simulations demonstrated relatively high turnover of the local populations, especially those that are small. This indicates that despite being a long-lived perennial, *S. hispanica* exhibits features of metapopulations, as we expected. The metapopulation framework (Hanski 1999) is thus a suitable approach for modelling the dynamics of *S. hispanica* and other similar species. More specifically, the high turnover of the small populations and high survival of the large ones suggests that *S. hispanica* is a likely representative of species with mainland–island metapopulation dynamics (Harrison 1991). From a conservation point of view, survival of large populations is crucial for survival of the whole metapopulation. These large populations may, however, be threatened in the landscapes by factors such as human-induced population destruction as well as an increased rate of herbivory.

Effect of herbivores on landscape-level dynamics

We expected *S. hispanica* to decline by the end of simulations using the current rate of herbivory because of (1) the negative effects of large herbivores on performance of *S. hispanica*, (2) its slow dynamics and dispersal-limitation (Münzbergová 2004) and (3) the increased fragmentation of the landscape over the last 60 years (Chýlová and Münzbergová 2008). Unexpectedly, our simulations of the prospects of *S. hispanica* following 30 years of current landscape conditions (herbivory and disturbance) suggest that the total number of individuals in the study area is largely stable. We can, however, expect slight declines in future habitat occupancy. *S. hispanica* was unable to establish new large populations during the simulations due to the high risk of extinction of small populations (as demonstrated also in the field by Münzbergová 2006).

The maintenance of landscape dynamics of *S. hispanica* near equilibrium (except the extinction of small populations) can be explained by the type of its local population dynamics. Population dynamics are very stable over time (even with the current high rate of herbivory) due to the high survival probability of individuals and occasional clonal reproduction. Nevertheless, a simulated 20% decline in the current herbivory rate markedly increased the number of seeds produced, resulting in higher seed dispersal and more successful colonization. In contrast, a 20% increase in the current rate of herbivory led to a serious population decline. This decline was due primarily to the large changes occurring in the most abundant populations. Sensitivity analyses of the dispersal parameters revealed that habitat occupancy depended partly on the estimation of the dispersal parameters. However, the dispersal parameters did not influence overall decline/increase of *S. hispanica* under higher/lower than current rates of herbivory. In addition, changes in dispersal rates had only negligible effects on landscape-level prospects of *S. hispanica* under high rates of herbivory. This indicates that the negative effects of herbivory on performance of species, specifically on generative reproduction (e.g., Knight et al. 2008; Ehrlén and Münzbergová 2009; Lin and Galloway 2009), can be much stronger than the

possible positive effects of herbivores as dispersal agents (Fischer et al. 1996; Nathan et al. 2008). The effect of herbivory was enhanced significantly when combined with population destruction (resulting from large disturbances). The importance of population destruction to species landscape dynamics has been previously demonstrated, for example by Münzbergová et al. (2005). Such a clear negative effect of herbivory and population destruction on species dynamics is caused by the absence of any positive effects of these activities on plant performance. If overcompensation (Paige and Whitham 1987) or enhanced seedling recruitment (Gomez 2005) was found in the case of *S. hispanica*, the effect of herbivory rate and population destruction on its landscape-level dynamics would be much less clear.

The current equilibrium state of the *S. hispanica* metapopulation may reflect several factors. One possibility is that the expansion of *S. hispanica* has been constrained in the past (e.g., by cattle grazing and mowing). This hypothesis is supported by the fact that many *S. hispanica* populations occur in former pastures (Münzbergová 2004). In such a scenario, current patch occupancy should reflect high past landscape connectivity. Alternatively, landscape connectivity may still be the same, but with an ongoing increase in the rate of herbivory and the frequency of large disturbances in the area. This would result in reduced growth rates of local populations and in higher probability of extinction of small populations.

Patterns of herbivory

First, it is important to note that the proportion of browsed individuals in a population does not necessarily relate to the frequency of visits by large herbivores, as the herbivores can readily pass over patches without browsing. We found less herbivore damage in larger patches and in patches surrounded by high shrub cover. This pattern corresponds to the results of previous studies of the habitat preferences of large herbivores. For example, roe deer prefer small patches (Aulak and Babinska-Werka 1990; Welch et al. 1990) with rich ground vegetation (Welch et al. 1990), and the density of deer increases with increasing habitat heterogeneity (Kie et al. 2002) and the density of habitat edges (e.g., Tufto et al. 1996; Saïd et al. 2005; Miyashita 2008). According to Lamberti et al. (2006), roe deer prefer open habitats (e.g., orchards and fields) to woodlands and scrublands. This latter observation suggests that the presence of shrubs or forest may decrease the attractiveness of patches to herbivores.

Another important factor affecting behaviour of large herbivores is human activity in the landscape, especially developed areas and roads. Several studies have found that these factors negatively impact deer density (e.g., Hewison et al. 2001; Coulon et al. 2008). In our study, populations near villages were browsed less heavily whereas the proximity of roads had the opposite effect. The increased rate of herbivory near roads may result from the use of roads as corridors by large herbivores. The affinity of large herbivores to individual patches may have been also affected by landscape topography and surface (Coulon et al. 2008). We found a higher rate of herbivory in the drier patches above valleys than in the wetter patches closer to valley bottoms.

Estimated parameters and model credibility

Several things should be kept in mind in interpreting our simulation results. The sensitivity analyses indicated that dispersal ability and carrying capacity had an effect on the total number of *S. hispanica* individuals under low, but not high, rates of herbivory. Similarly, these parameters had a greater effect on habitat occupancy under low than under high herbivory rates (Supplementary Data Figs S1 and S2). Similar results were found when using smaller initial population sizes and running the simulations for 100 years (data not

shown). These results indicate that our conclusions regarding the effects of large herbivores on the species landscape dynamics are independent of the parameter estimates.

To simulate the prospects of *S. hispanica* in the landscape, we set the initial patch occupancy at 74%, corresponding to the observed occupancy of this species in the study area. This occupancy level is significantly higher than the 32% found by Münzbergová (2004), who used sowing experiments to identify patches suitable for, but unoccupied by, *S. hispanica*. However, in the study by Münzbergová (2004) patches were distributed over a larger area (approx. 400 km^2) than in our study (approx. 20 km^2), but within the same landscape. In addition, in the study of Münzbergová (2004), the predictions of patch suitability were based on seedling establishment, which does not necessarily reflect patch suitability for adult plants (Ehrlén et al. 2006). In our study, suitable patches were identified using a combination of abiotic conditions and species composition. These types of factors have been shown previously to explain species distribution (e.g., Dupré and Ehrlén 2002; Münzbergová 2004; Ehrlén et al. 2006; Chýlová and Münzbergová 2008). As suggested, for example, by Tájek et al. (2011) and observed in the present study, the combination of these two types of factors provides the best predictions of habitat suitability for a species. Specifically, we identified drier, shaded, basic, nutrient-rich patches as more suitable for *S. hispanica* in dry grasslands. Patch preference may reflect both specific abiotic conditions and past land use (particularly as pastures). Nevertheless, the sensitivity analyses revealed that a reduction in the number of suitable patches had little effect on the model results.

In our simulations, local population dynamics was assumed to be the same among patches (except for the impact of herbivory) and largely stable over time. We simulated demographic stochasticity representing random changes in local population dynamics over time. There was no indication for a gradual change in environmental conditions. In our simulations we thus assumed that among-year variation in the local population dynamics was caused only by environmental stochasticity. Environmental stochasticity was simulated by drawing a random transition matrix (for each population, in each step) from a set of matrices. The matrices were very similar and thus their random sample had little effect on the local population dynamics. Although differences between patches and changes in local population dynamics over time could occur, we argue that these factors are unlikely to strongly affect our conclusions. First, all our populations occur within a small area under very similar habitat conditions, minimizing potential differences among populations. Second, our simulations extended only 30 years into the future, making it unlikely that habitat conditions will change dramatically. Third, it is likely that the responses of *S. hispanica* to any landscape changes would be slow, and thus minimal over this period. Therefore, browsing by large herbivores is probably the primary factor influencing the prospects of *S. hispanica*.

Finally, we assumed a stable rate of herbivory at each patch during the individual simulations. However, the incidence of browsing by large herbivores fluctuates between years due to changes in their abundance. For example, 22% more roe deer were recorded in the landscape in 2008 than in 2007 (Municipality Litoměřice, Department of Environment). However, we suggest that fluctuations in herbivory rate are unlikely to alter the main conclusions of the model.

Conclusions

Our field observations indicate that over 60% of flowering *S. hispanica* individuals are damaged by large herbivores in most populations each year. Our simulations, however, suggest that current dynamics of *S. hispanica* are approximately in equilibrium under the current rate of herbivory and frequency of large disturbances (one per 30 years per

population). The simulation results also revealed a higher survival probability of large populations than that of small ones. Therefore, under current landscape conditions, the prospect of *S. hispanica* in the landscape depends heavily on the prospects of large populations.

Simulations of the effect of herbivory rate on the dynamics of *S. hispanica* indicated that a decline or increase in the herbivory rate of more than 20% over the entire landscape could lead to a rapid expansion or decline of the species. This effect is predicted to be much stronger under the additional occurrence of disturbance. These results confirm our hypothesis that browsing by large herbivores can have dramatic effects on the landscape dynamics of species if important components of the life cycle are strongly affected by these herbivores.

Finally, as concluded in other studies, our study suggests that the probability of population destruction should be incorporated into models predicting changes in species distributions. Incorporating the effect of large herbivores and population destruction into models of species landscape dynamics should be a major endeavour of future metapopulation studies.

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Appendix S1. – Collecting and testing the data on the impact of large herbivores on performance of *S. hispanica*.

In 2009 and 2010, Zita Červenková conducted a field demographic study of one large *S. hispanica* population (no. 16 in Table 1 in Münzbergová 2006). Two sets of tagged flowering plants were compared. The first set was exposed to browsing by large herbivores (140 individuals), whereas the second set was caged and thereby fully protected from browsing (70 individuals). In both seasons, Z. Červenková recorded plant height and flowerhead number; in the plants exposed to browsing, she also recorded the presence of herbivory (1/0). Mature seeds of all plants were collected and counted. For the exposed plants, Z. Červenková collected performance data only from those plants that experienced herbivory (119 individuals). Z. Červenková then compared the performance of browsed and intact individuals. Specifically, she tested for differences in seed production, the production of flowering or vegetative clones, transitions from flowering to vegetative stages and the stasis of flowering individuals.

Z. Červenková identified two transitions that were significantly affected by browsing. First, clonal reproduction of flowering stalks was absent among browsed plants and occurred in 10% of intact plants (df error = 185; F = 14.64; P = 0.001). Second, she observed significant differences between browsed and intact plants in seed production. Intact plants produced an average of 92.0 ± 6.2 SE seeds, while plants damaged by large herbivores produced only 4.3 ± 1.2 SE seeds per plant (df error = 185; F = 314.39; P < 0.001). There was no significant difference between browsed and intact plants in the transition stasis of flowering plants (df error = 185; χ^2 = 214.70; P = 0.93), in the transition from flowering to large vegetative plants (df error = 185; χ^2 = 211.50; P = 0.90) nor in the production of vegetative clones (df error = 185; F = 0.65; P = 0.42).

Appendix S2. – Detailed description of the landscape-level model.

Simulations are performed on a grid representing a study area, with suitable patches explicitly located and surrounded by unsuitable areas. Multiple cells can be coded as part of the same patch. To simulate the population dynamics of a species, a population vector containing the number of individuals in each size class is assigned to each patch. Population growth at the *k*-th patch is simulated using transition matrices as

$$x_k'(t+1) = A_{Q(k)} \times x_k(t),$$

where x_k is a population vector on patch *k* at time *t*, and A_Q is a transition matrix of quality *Q* (e.g., corresponding to the rate of herbivory at patch *k*). Density dependence within patches is modelled using a logistic growth equation; the difference between total (N) and maximum (K) population size at each patch is used as the logistic term. The population vector $x_k'(t+1)$ resulting from the matrix multiplication is then replaced by the following term:

$$x_k''(t+1) = x_k'(t+1)\frac{1}{\lambda}\left[1+(\lambda-1)\left(1-\frac{N_k(t)}{K_k}\right)\right],$$

where λ is the dominant Eigenvalue of the matrix A_Q, $N(t)$ is the total population size (sum of all stages) at patch *k* at time *t* (before the matrix multiplication) and *K* is the capacity of patch *k* (i.e. the maximum number of individuals at the patch of the given size). This form of density-dependence affects all transitions equally. Demographic stochasticity is simulated by replacing each vector element by a Poisson-distributed deviate with a mean of x_{ik}''. A new population vector $x_k(t+1)$ is created by combining these numbers.

Environmental stochasticity is simulated using several matrices for each habitat quality. At each step, one matrix from a set of matrices assigned to the given patch is randomly chosen with a probability assigned to the matrix. Environmental stochasticity is assumed to be uncorrelated over space and time; the random matrix is therefore chosen independently for each patch. Such an assumption could be used in case of *S. hispanica*, because there was very small among-year and among-population variation in the transition matrices. The choice of specific matrix has little effect on the population dynamics at the patch.

The model simulates distance-dependent and distance-independent dispersal. For distance-dependent dispersal, seeds produced at each patch are divided equally among the cells that comprise that patch and dispersed from these cells according to either a negative exponential function, or a negative hyperbolical function, or their summation. Distance-independent dispersal is simulated by removing a fraction of seeds at each step from each patch, then randomly redistributing them over the whole simulation plane. The seeds arriving at all cells within a patch are summed to yield the seed input at that patch. Seeds that fall into the space between patches are considered lost. Model setting enables either absorbing or periodic boundaries of the simulation plane, such that diaspores passing over the boundary are either lost or appear across the plane, respectively. At each step, transitions within patches are simulated to yield a seed crop before performing any dispersal.

Table S1. – List of selected dry grassland species.

Agrimonia eupatoria	*Gymnadenia conopsea*
Anemone sylvestris	*Helianthemum nummularium subsp. grandiflorum*
Anthericum ramosum	*Hieracium pilosella*
Anthyllis vulneraria	*Inula salicina*
Asperula cynanchica	*Laserpitium latifolium*
Asperula tinctoria	*Leontodon hispidus*
Aster amellus	*Linum flavum*
Aster linosyris	*Linum tenuifolium*
Astragalus cicer	*Listera ovata*
Astragalus glycyphyllos	*Lotus corniculatus*
Brachypodium pinnatum	*Medicago falcata*
Bromus erectus	*Melampyrum arvense*
Bupleurum falcatum	*Melampyrum cristatum*
Campanula glomerata	*Melampyrum nemorosum*
Campanula rotundifolia	*Ononis spinosa*
Carex flacca	*Peucedanum cervaria*
Carex humilis	*Plantago media*
Carex tomentosa	*Potentilla arenaria*
Carlina vulgaris	*Potentilla heptaphylla*
Centaurea jacea	*Prunella grandiflora*
Centaurea rhenana	*Salvia pratensis*
Centaurea scabiosa	*Salvia verticillata*
Cirsium acaule	*Sanguisorba minor*
Cirsium eriophorum	*Seseli hippomarathrum*
Cirsium pannonicum	*Sesleria albicans*
Coronilla vaginalis	*Stachys recta*
Coronilla varia	*Tanacetum corymbosum*
Eryngium campestre	*Thesium linophyllon*
Festuca rupicola	*Thymus praecox*
Fragaria viridis	*Trifolium medium*
Gentiana cruciata	*Trifolium montanum*
Geranium sanguineum	*Veronica austriaca subsp. teucrium*
Globularia elongata	

Note: Nomenclature follows Tutin et al. (1964-1983).

Table S2. – Correlation matrix of habitat characteristics recorded at dry grassland patches. Significant correlations ($P < 0.05$) are in **bold**.

		Log (area)	Slope	TWI	PDSI De	PDSI Ja	PDSI Fe	PDSI Ma	PDSI Ap	PDSI Ma	PDSI Ju	Beals index	Ellenberg indicator values Light	Moist	Soil re	Nutr	Temp	Cont	Fo 1 km	Sh 1 km	Fo 0.5 km	Sh 0.5 km	Near fo	Near sh	Near vill
Slope		-0.02																							
TWI		0.09	**-0.65**																						
PDSI	De	**0.23**	-0.18	0.15																					
	Ja	**0.24**	-0.21	0.17	**1.00**																				
	Fe	**0.23**	**-0.25**	0.21	**0.99**	**1.00**																			
	Ma	**0.23**	**-0.30**	**0.24**	**0.99**	**0.99**	**1.00**																		
	Ap	**0.22**	**-0.38**	**0.28**	**0.97**	**0.98**	**0.99**	**1.00**																	
	Ma	**0.22**	**-0.48**	**0.34**	**0.94**	**0.95**	**0.97**	**0.98**	**0.99**																
	Ju	0.21	**-0.54**	**0.38**	**0.91**	**0.93**	**0.95**	**0.96**	**0.98**	**1.00**															
Beals index		**0.25**	**0.43**	**-0.32**	-0.20	-0.21	**-0.23**	**-0.25**	**-0.28**	**-0.32**	**-0.34**														
Ellenberg indicator values	Light	**0.48**	-0.02	-0.08	**0.25**	**0.24**	**0.24**	**0.24**	**0.24**	**0.23**	**0.22**	-0.03													
	Moist	**-0.28**	-0.10	**0.23**	-0.20	-0.20	-0.20	-0.19	-0.18	-0.15	-0.14	**-0.47**	**-0.30**												
	Soil re	**0.26**	**0.31**	-0.18	-0.05	-0.05	-0.07	-0.08	-0.10	-0.13	-0.15	0.21	**0.35**	-0.03											
	Nutr	-0.10	**-0.28**	0.19	0.16	0.16	0.18	0.19	0.21	**0.24**	**0.25**	**-0.60**	0.13	**0.56**	**-0.24**										
	Temp	**0.25**	**-0.38**	0.20	**0.52**	**0.52**	**0.54**	**0.55**	**0.57**	**0.58**	**0.58**	-0.09	0.16	-0.19	**-0.27**	0.10									
	Cont	-0.02	**-0.24**	-0.03	0.17	0.17	0.18	0.19	0.21	**0.22**	**0.23**	**-0.28**	0.12	**-0.28**	-0.07	-0.13	**0.37**								
Fo 1 km		-0.15	**-0.35**	**0.36**	**0.25**	**0.25**	**0.26**	**0.27**	**0.30**	**0.33**	**0.35**	-0.09	**-0.31**	**0.23**	**-0.27**	0.19	0.20	-0.12							
Sh 1 km		0.16	0.11	-0.09	-0.16	-0.16	-0.16	-0.16	-0.16	-0.17	-0.17	**0.22**	-0.21	0.07	-0.21	-0.16	**0.24**	-0.09	0.05						
Fo 0.5 km		**-0.25**	**-0.38**	**0.39**	0.01	0.03	0.04	0.06	0.09	0.14	0.17	-0.09	**-0.41**	0.18	**-0.26**	0.08	0.05	-0.07	**0.83**	-0.08					
Sh 0.5 km		**0.30**	0.16	-0.08	-0.11	-0.12	-0.12	-0.13	-0.14	-0.15	-0.16	0.10	0.02	0.08	0.06	-0.07	0.10	-0.14	-0.11	**0.70**	**-0.24**				
Near fo		0.21	0.20	-0.09	0.00	0.01	-0.01	-0.02	-0.04	-0.06	-0.08	-0.07	**0.26**	-0.01	**0.31**	-0.07	-0.18	0.06	**-0.55**	-0.20	**-0.59**	-0.06			
Near sh		0.01	-0.21	**0.29**	0.19	0.19	0.19	0.20	0.21	**0.23**	**0.25**	0.01	-0.02	-0.08	-0.16	-0.02	0.06	-0.03	**0.45**	**-0.40**	**0.56**	**-0.38**	**-0.22**		
Near vill		-0.13	-0.20	**0.22**	**-0.23**	**-0.23**	**-0.23**	**-0.21**	-0.19	-0.16	-0.13	-0.03	**-0.32**	0.13	**-0.27**	-0.04	-0.04	-0.10	**0.37**	**0.26**	**0.43**	**0.34**	**-0.27**	0.19	
Near ro		0.07	-0.10	0.18	-0.11	-0.11	-0.10	-0.09	-0.08	-0.06	-0.04	-0.02	**-0.21**	0.08	**-0.22**	-0.11	0.07	-0.15	0.19	**0.40**	**0.26**	**0.50**	-0.14	0.17	**0.70**

Abbreviations: Log = logarithm; TWI = topographic wetness index; PDSI = potential direct solar irradiation; De-Ju = December-June; Moistr = moisture; Soil re = soil reaction; Nutr = nutrients; Temp = temperature; Cont = continentality; Fo = forest; Sh = shrub; Near = nearest; vill = village; ro = road.

Fig. S1. – Sensitivity analysis of the model: the negative effect of increasing herbivory rates on the total number of *S. hispanica* individuals in the simulation area at the end of simulations, using different values of the model parameters: a) distance-dependent dispersal (*D*), b) distance-independent dispersal (*p*), and c) maximum population density (*K*). Mean ± SD are shown for all simulations. Initial model parameters were set at $D = 2.51$ m, $p = 0.001$, $K = 0.97.m^{-2}$. "Predicted" shows the current rate of herbivory predicted in each patch in the area. The rate of herbivory was increased or decreased incrementally by 10% for all patches until it reached 100% or 0%, respectively.

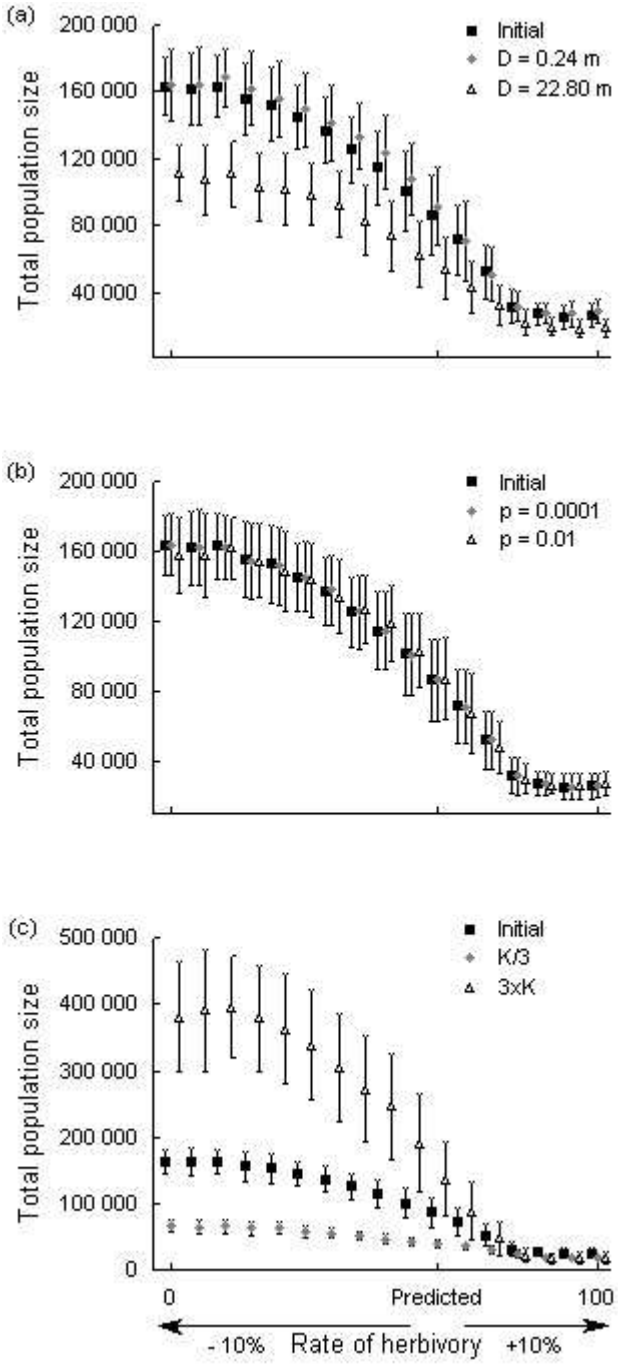

Fig. S2. – Sensitivity analysis of the model: the negative effect of increasing herbivory rates on patch occupancy by *S. hispanica* in the simulation area at the end of simulations, using different values of the model parameters: a) distance-dependent dispersal (*D*), b) distance-independent dispersal (*p*), and c) maximum population density (*K*). Mean ± SD are shown for all simulations. Initial model parameters were set at D = 2.51 m, p = 0.001, K = 0.97.m^{-2}. "Predicted" shows the current rate of herbivory predicted in each patch in the area. The rate of herbivory was increased or decreased incrementally by 10% for all patches until it reached 100% or 0%, respectively.